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A PRIMARY CAUSE OF DARKENING IN BOILED POTATOES AS REVEALED BY GREENHOUSE CULTURES[1]

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INTRODUCTION

Potatoes that darken after boiling have become increasingly prevalent in Wisconsin in recent years. When blackening in boiled potatoes (*Solanum tuberosum*) first became the subject of study in the fall of 1932 it was thought that some deficiency in soil minerals might be involved, and that this deficiency was probably linked with climatic influences upon both the soil and the plant. Accordingly, a summary of average weather conditions representative of northern (Antigo), central (Hancock), and southern (Madison) Wisconsin for the greater part of the preceding 5-year growing seasons was assembled. Table 1 shows the departures from the normal temperature and precipitation for each of these periods. From these data it is apparent that the growing season for the 3 years 1930–32 was subject to high temperature while the rainfall was generally deficient in all but the southern area. The records for August and September as related to periods of active tuberization are presented in table 2. The records for August show uniformly unfavorable departures from normal in 1930, with Hancock subject to drought in 1931 and Antigo to heat in 1932. The records for September show that this month was excessively dry in Hancock in 1930, excessively hot and wet in all areas in 1931, and droughty in all areas in 1932. The last-named year was the beginning of an era characterized by hot, dry summers which extended through 1939. During this period the discoloration of potatoes after boiling was more evident than before.

TABLE 1.—*Departures from the normal temperature and precipitation for the growing seasons 1928–32, as shown by the monthly averages, June to August, inclusive, at stations representative of northern (Antigo), central (Hancock), and southern (Madison) Wisconsin*

Year	Antigo		Hancock		Madison	
	Temperature	Precipitation	Temperature	Precipitation	Temperature	Precipitation
	°F.	*Inches*	°F.	*Inches*	°F.	*Inches*
1928	−1.0	+2.13	−2.2	+1.34	−1.5	+0.9
1929	−.5	−.12	−2.0	+.59	−1.6	+.8
1930	+2.4	−.39	+.7	−.51	+1.3	−.1
1931	+3.2	−1.16	+3.0	−.66	+3.0	+.2
1932	+2.7	−1.71	+2.6	−.59	+2.1	0

[1] Received for publication September 1, 1942. Contribution from the Department of Biochemistry, College of Agriculture, University of Wisconsin.

TABLE 2.—*Departures from normal temperature and precipitation for periods of tuberization at stations representative of northern (Antigo), central (Hancock), and southern (Madison) Wisconsin, 1928–32*

Year and month	Antigo		Hancock		Madison	
	Temperature	Precipitation	Temperature	Precipitation	Temperature	Precipitation
	F.	*Inches*	*F.*	*Inches*	*F.*	*Inches*
1928:						
Aug.	+1.9	+3.62	+0.6	+2.78	+0.4	+1.5
Sept.	−3.8	+2.73	−4.6	+1.07	−3.4	−1.94
1929:						
Aug.	+.1	−.83	−2.0	−1.11	+1.3	−2.3
Sept.	+1.7	−.96	−1.6	−1.12	−1.8	−.55
1930:						
Aug.	+3.5	−2.25	+1.5	−2.39	+2.7	−1.6
Sept.	+1.4	−.46	+.4	−2.52	+1.4	+1.07
1931:						
Aug.	+.2	−.16	+.4	−.99	+1.0	+2.0
Sept.	+6.5	+1.82	+4.9	+2.95	+6.0	+3.45
1932:						
Aug.	+3.4	−.75	+1.3	+.32	+1.4	−
Sept.	−.7	−1.56	−1.1	−1.66	−.5	−3.54

GENERAL PROCEDURE

The greenhouse experiments described in the present paper were undertaken to supplement observations on the prevalence of tuber discoloration in relation to climatic and fertility variations in the field.

The usual procedure was to break the dormancy of the tubers by heat treatment. This was done usually in December and tuber cuttings were planted in flats of pure quartz sand. Unless specified otherwise, the seed stock was obtained as free from disease as possible by the aid of associates engaged in its certification.[2] As soon as sprouting was general the plants were exposed to natural illumination and supplied with small amounts of the major nutrient elements. At this stage of development, and for several weeks after transplanting, the house was kept cool (below 60° F. at night) to stimulate vigorous vegetative development. Thus the plants available for transplanting were fairly uniform, and the major vegetative activity occurred at a time when increasing intensity of sunlight and length of day were favorable. The maturation of the crop occurred largely in April, while outdoor temperatures were still favorably low. During the early development of the crop the length of day was increased somewhat by operating lamps through an electric time switch, but the intensity of this illumination at the general plant surface did not exceed 200 foot-candles. Shortening of the daylight period was accomplished for the bed cultures by the use of canvas covers. Values of the solar radiation during the several culture periods, based on records of climatic conditions provided by the local United States Weather Bureau office one-half mile from the greenhouse, are given in table 3.

As the work progressed the writers became aware of the need of storing the crop for about 1 month after harvesting before making the blackening test. This interval allows time for the gradual transformations which cause discoloration after cooking. It was also

[2] For this assistance the writers are indebted to Professors J. G. Milward, John W. Brann, and G. H. Rieman, of the Departments of Horticulture, Plant Pathology, and Genetics, respectively.

found that halved tubers boiled in the skins should be peeled before grayness was determined in order to allow lateral diffusion of light into the tissue and prevent its absorption. Therefore, all of the boiling records obtained from longitudinal halves of tubers, which antedated the autumn of 1939, have been adjusted in accordance with this finding.

TABLE 3.—*Values of outdoor solar radiation during the several culture periods in the greenhouse bed and in pots*

Year	Period	Greenhouse bed			Pots		
		Total solar radiation per cm.[2]	Proportion of normal	Proportion of the June-August normal	Total solar radiation per cm.[2]	Proportion of normal	Proportion of the June-August normal
		Gram calories	*Percent*	*Percent*	*Gram calories*	*Percent*	*Percent*
1934	Feb. 1–May 14	35,186	104	77			
1935	Mar. 15–June 15	36,092	92	79			
1936	Jan. 15–Apr. 15	26,188	106	57			
	Feb. 1–Apr. 20				24,660	105	54
1937	Jan. 6–Apr. 20	29,349	104	61	29,349	104	61
1938	Jan. 1–Apr. 15				23,639	88	52
	Feb. 1–Apr. 15	19,831	90	43			
1939	Jan. 16–Apr. 20				27,110	103	59

No particular refinement was applied to the boiling procedure, for it was observed that the proportion of water used, its hardness, and the time of boiling had little influence on the final discoloration. The samples were removed before they showed any tendency to disintegrate, and full discoloration occurred in the process of cooling. Ten tubers selected at random in one test were found to give reproducible results, but a later trial has indicated that a larger sample may be necessary for full reliability of the boiling record. The cooked samples were observed against a background of ordinary filter paper under northern skylight and the discoloration is expressed as light gray and medium gray, It was impracticable to compare the grayness of the mashed tissue with photometric standards, as was done by Bilham and associates (*1*),[3] because the preponderant white particles of starch cover the darkly pigmented products.[4] Darkening after boiling is common to regions adjacent to mechanical injury or pathological invasion and therefore bruised or scabby potatoes have been excluded from the samples.

BED PLANTINGS

SEASON OF 1934

A soil medium was prepared in a bed 7 inches deep by mixing about 1 part by weight of Miami silt loam with 4 parts of sand from a local deposit. From data on the composition of these soils it was estimated that the mixture contained not more than 0.05 percent nitrogen, 0.02 percent phosphorus, and 0.50 percent potassium, with 70 pounds of available potassium per acre. This soil lay upon the clay subsoil at the bottom of the bed, so that exchanges of water and nutrients between the two were possible. No fertilizer was applied

[3] Italic numbers in parentheses refer to Literature Cited, p. 193.

[4] This explanation was suggested by Edward E. Miller, formerly research assistant, Department of Physics, University of Wisconsin.

but a differential of water supply in parts of the bed was established during the last month of culture.

In the 1934 test two discoloring seed stocks of Rural New Yorker were compared with a normal one. One of the abnormal stocks had turned quite dark after cooking and the other was classified as "weak." The latter had internal browning and, on cooking turned a bluish black. Sprouts emerged from the normal stock much later than from the abnormal ones and the stems of the latter were somewhat spindling. No artificial illumination was applied to this crop.

The yields were substantial from the abnormal stocks but low from the normal one, with general benefit from irrigation. As the boiling test was made soon after the crop was dug, the significance of the result is questionable. All the samples rated acceptably white as of that time.

SEASON OF 1935

In the 1935 test emphasis was placed upon deficiency of potassium in association with drought. The residual soil of the previous season was treated with a general fertilizer about the equivalent to 1,200 pounds to the acre of 3–10–20, in the complete form, which provided a liberal supply of potassium. On one-half the area the added potassium was decreased 90 percent by replacing potassium sulfate with its equivalent of sodium sulfate. Nitrogen was supplied in ammonium sulfate and phosphorus in dicalcium phosphate. The general plan of treatment was as follows:

Low K supply		High K supply	
Droughty	Irrigated	Droughty	Irrigated

Five hills of the following seed stocks were planted in each section: Irish Cobbler, Green Mountain, Katahdin, certified Rural New Yorker, and a stock of Rural New Yorker selected by test as darkening after boiling. The size of bed permitted planting distances of 1.5 feet between rows and 1.0 foot between hills. This density of stand appeared to be justified by the limiting light intensity of the culture period. Plants from the abnormal Rural New Yorker seed stock appeared about 3 weeks later than those from the other stocks. Restriction of water supply on the designated areas was begun about a month before harvesting.

The yields, on the basis of 3-foot spacing between hills and rows, ranged approximately from 16 to 160 bushels per acre. Low supplies of both water and potassium produced tubers with peaked ends, but this effect was noticeable also where the potassium supply was restricted and the water supply liberal. Slenderness of potatoes in relation to potassium deficiency has been observed by Martin et al. (*4*). The crop was stored at 50° to 60° F. for 1 month before it was tested for color after boiling. Light grayness was confined to tubers produced by the Green Mountain and normal Rural New Yorker stocks in the droughty area on the low-potassium treatment. No correlation was found between grayness and shape of tuber. The crop from discoloring seed stock was free from grayness.

SEASON OF 1936

Differentials of water and potassium similar to those of 1935 were supplied. Since there was an accumulation of potassium in the low potassium areas the used soil was replaced by a mixture similar

to that used in 1935. This contained 125 and 75 pounds, respectively, of available K and P per acre, and had a pH value of 7.0. Its water-holding capacity was 27 percent. The application of complete fertilizer was equivalent to 1,200 pounds of 4–10–12 per acre, with 60 percent of the nitrogen in organic form. There was one-half as much available potassium in the control plot and no use was made of sodium as a substitute. After mid-March water was withheld from parts of the bed, and on April 6 the soil of the droughty and irrigated sections contained 2.1 and 8.0 percent (dry-weight basis), respectively; these values correspond to about 8 and 30 percent of saturation.

The potatoes were stored at 50° to 60° F. for about 1 month before boiling. The only samples that discolored were of the Burbank variety produced on the low-potassium areas, both under irrigation and exposed to drought. The range in yield was the same as in 1935. No correlation was noted between yield and degree of darkening after cooking; that is, there was no evidence of depletion of nutrients by high yields attended by tuber discoloration.

SEASON OF 1937

The primary purpose of the 1937 planting was to test the capacity of certain rarer mineral elements to prevent discoloration of potatoes produced with a relatively low supply of potassium. Burbank stock, which had been propagated at Hancock from high-grade seed stock obtained from Idaho in 1935, was used.

The residual soils in the bed sections now contained 125 to 150 pounds of available phosphorus per acre. Adjustments of the potassium content were made to give 150 pounds per acre in two of the sections and 275 pounds in the others. The pH was about 7.8 and the nitrate content was low. Two weeks after transplanting with seed pieces attached, two sections at each level of potassium supply were given an application of hydrated manganous chloride and boric acid at rates of 20 and 10 pounds per acre, respectively. Ammonium salt was applied from time to time until it reached a total of 80 pounds per acre. Shortening of the day was begun March 1 and 3 weeks later there was some fading of crown leaves, although tip growth was vigorous. This effect was especially noticeable on the low-potassium areas and may have resulted from subnormal utilization of nitrogen.

The crop yield was as high as 250 bushels per acre. The tubers were stored for about a month at 60° F. before they were tested, and many of the mature ones had previously been exposed to relatively high temperatures in the bed. Because of these conditions it was believed that the cooking test would give significant results. The results are shown in table 4.

It is worthy of note that tubers of the Burbank variety from all cultural treatments discolored when boiled while all tubers of the Chippewa variety cooked white. The Irish Cobbler discolored more generally than the Rural New Yorker, and developed on the low-potassium supply supplemented by rarer elements the localized intensity of discoloration in the stem end or cortical region which is characteristic of the most seriously affected potatoes. This experiment gives no consistent indication of cause and effect between fertilizer treatments and postcooking pigmentation of the susceptible varieties.

TABLE 4.—*Effect of a low supply of potassium with rarer elements added on yields and on discoloration of potatoes grown in a greenhouse bed, 1937, and effect of heat and drought with rarer elements limited or ample, 1938*

SEASON OF 1937

Variety	Water	Potassium	Rarer elements	Weight of fresh tubers	Boiling record [1]
				Grams	
Burbank	Ample	Low	None	781	LG
			Manganese and boron	778	MG
		High	None	1,510	LG
			Manganese and boron	1,219	LG
Chippewa	do	Low	None	722	W
			Manganese and boron	814	W
		High	None	1,405	W
			Manganese and boron	1,241	W
Irish Cobbler	do	Low	None	620	LG
			Manganese and boron	536	MG
		High	None	888	LG
			Manganese and boron	1,006	W
Rural New Yorker	do	Low	None	132	W
			Manganese and boron	207	W
		High	None	637	LG
			Manganese and boron	423	LG

SEASON OF 1938

		Soil heating during tuberization	Boron, copper, manganese, and zinc		
Chippewa	Restricted late	Applied	Limited	220	W
			Ample	49	W
		None	Limited	172	W
			Ample	171	W
Irish Cobbler	do	Applied	Limited	193	LG
			Ample	98	W
		None	Limited	131	LG
			Ample	167	LG
Rural New Yorker	do	Applied	Limited	21	W
			Ample	37	LG
		None	Limited	16	W
			Ample	36	W
Triumph	do	Applied	Limited	54	W
			Ample	119	W
		None	Limited	22	W
			Ample	58	LG

[1] W=white; LG=light gray; MG=medium gray.

SEASON OF 1938

In view of the failure to induce darkening after cooking consistently by fertility deficiencies and drought in the bed cultures of previous years, attention was directed in 1938 to another climatic factor, heat. An electric heating cable was installed [5] in one-half of the bed at a depth of about 3 inches from the surface of the soil. The unit was so distributed as to fall between hills in the rows, its operation being controlled by the combined use of a time switch on the electric service and a thermostat with its expansion element buried in the soil.

An examination of the residual soil showed that the soil reaction was uniform (pH 7.5) throughout the bed sections, as was also the distribution of ammoniacal nitrogen (5 pounds per acre). Dicalcium phosphate and sodium nitrate were added in quantities to give uniform values of 50 pounds per acre of nitrate and 85 pounds per

[5] Courtesies extended by representatives of the General Eelectric Co. in connection with the installation are appreciatively acknowledged.

acre of available phosphorus. The supply of available K was brought to 125 pounds per acre by the addition of potassium sulphate. It was assumed that the sections treated with manganese and boron in 1937 would retain sufficient residues of these elements; applications of 5 pounds per acre each of the hydrated sulfates of copper and zinc were added to these sections. Several weeks after planting, an additional 50 pounds per acre of ammonium salt was applied to all sections. The seed pieces were cut to approximate a 1-inch cube about each eye. When transplanted to the bed the Rural New Yorker had a very poorly developed root system.

The heater was put into operation from 8 a. m. to 4 p. m. on April 1, when the use of the lamps for controlling length of day was discontinued. The use of the heater was limited to 2 weeks because of the rapid maturity of the plant tops. On days when the outdoor radiation was about one-half to two-thirds normal the temperature differential between heated and unheated portions of the bed 3 inches below the surface was about 7° F. at midday, or an interval of 86° to 79° F. Immediately after the heater was turned on the soil of the entire bed became dry. Determinations on April 9 showed the water content in the heated portion of the bed to be 2.4 percent where boron, copper, managnese, and zinc were limited and 2.6 percent where these elements were ample; in the part of the bed where heat was not applied the water content was 2.7 percent where these elements were limited and 4.9 percent where they were ample. It may be supposed that sheltering from direct sunlight by the southern end wall of the house contributed to a greater retention of water in the last section. Table 3 shows that the season was unusually cloudy.

The boiling test was made after the tubers had been stored for 1 month at about 60° F. The test showed that heat and drought, operating simultaneously, and primarily on the developing tuber, had no detectable influence upon darkening after cooking (table 4). However, the vines were made somewhat independent of drought by the practice of frequently sprinkling the unoccupied floor of the house. In this experiment Irish Cobbler was most generally subject to discoloration while Chippewa remained white throughout the tests. There is no evidence that the application of rarer elements was beneficial so far as whiteness of the cooked tubers was concerned,

POT CULTURES

Pot culture was adopted primarily for the purpose of isolating the plants from influences of the subsoil in the greenhouse bed. The containers most frequently used were galvanized-iron boxes, 1 foot square and 8 inches deep, adapted to the use of 50 pounds of sand. To protect against contamination, the inside of the boxes was coated with paraffin. Ottawa silica sand, screened to 30–40 mesh and analyzing about 99.5 percent SiO_2, served as the culture medium.

In terms of commercial fertilizer, the regular nutrient mixture supplied approximated 1,000 pounds per acre of a formula which ranged from 6.5–15–15 in 1936 to 2–18–23 in 1938. Appropriate amounts of the minor nutrient elements were added in various combinations to some cultures. The proportions of salts used are shown in table 5, and the variations in the supply of potassium and boron are given in the text. An application of 1.0 gm. per pot approximates the equiv-

TABLE 5.—*Nutrient applications to pot cultures* [1]

Salt applied	1936	1937	1938	1939
	Gram	*Gram*	*Gram*	*Gram*
Dicalcium phosphate, hydrated	5.0	5.0	5.0	5.0
Magnesium nitrate, hydrated	5.0	3.0	3.0	3.0
Potassium sulfate	4.0	4.0	5.0	4.0
Ammonium sulfate	[2] 3.0	2.4	1.0	1.4
Ferric citrate	.3	.3	.3	.3
Manganous sulfate, hydrated	.03	.025	.025	.025
Boric acid	[3] [4] .0006	.0005	.030	.030
Copper sulfate, hydrated	[4] .0003	.005	.030	.005
Zinc sulfate, hydrated	[4] .0003	None	.015	.005

[1] See text for departures from normal.
[2] Ammonium chloride used instead of ammonium sulfate.
[3] Borax used instead of boric acid.
[4] Estimated amount.

alent of 100 pounds per acre, on the area basis. Phosphorus and iron carriers were added before transplanting and the rarer mineral elements and part of the magnesium salt were added immediately after transplanting. The remainder of the major elements were added as the plants developed. With the shift of proportions between calcium and magnesium salts the proportion between molecular equivalents of calcium and magnesium oxides was increased from about 2.0:1.0 to 3.5:1.0.

The water content of the soil was controlled by weighing the containers and contents when watering appeared necessary. In this way wide differences were maintained in the water content of different cultures. The plants in the pot experiments were subjected to the same use of artificial illumination as those in the beds, but they were not covered for shortening the day length as maturation approached.

SEASON OF 1936

In the 1936 experiments the salt applications were varied to provide different levels of potassium carriers (2, 4, and 6 gm.), and these were associated with similar variations in the accompanying sulfur supply. A separate group of cultures of Rural New Yorker received liberal proportions of calcium with the highest level of potassium. The calcium was supplied in two forms, one (hydrated calcium sulfate, 6.8 gm. per pot) to provide for mass effects in its proportion to potassium and other elements and the other (calcium carbonate, 8.0 gm. per pot) to simulate the condition of reduced availability of other mineral elements brought about by change of reaction in the liming of soil. The pH of the culture medium, as determined during the late vegetative activity of the plants, seemed too little influenced by the form of calcium supplied to be an important factor in the availability of mineral elements. Leachings of the medium to which calcium carbonate was added had a pH of 6.3, and those from the cultures supplied calcium sulfate had a pH of 5.9, as determined by the colorimetric method.

One month after transplanting marked differences were apparent in the development of the tops of different plants and between some treatments of the Rural New Yorker. The plants not given the extra application of calcium salts had rather slender stems, those receiving calcium sulfate were short and much branched, while those receiving calcium carbonate were both tall and rugged. Terminal buds of plants in the group treated with calcium sulfate showed desiccation

and fading in a manner indicative of toxicity from excess calcium. By the middle of March yellowing of the basal leaves in many cultures had become conspicuous, but this effect showed no correlation with low supplies of potassium. The addition of the third gram of nitrogen carrier at that time was followed by partial recovery.

By April the leaves of the low-potassium cultures of Rural New Yorker showed a tendency to roll, but the severity of the leaf roll decreased as the supply of potassium increased (fig. 1). Although the effects of potassium deficiency were less severe on Triumph than on Rural New Yorker, the former variety suffered some bleaching of

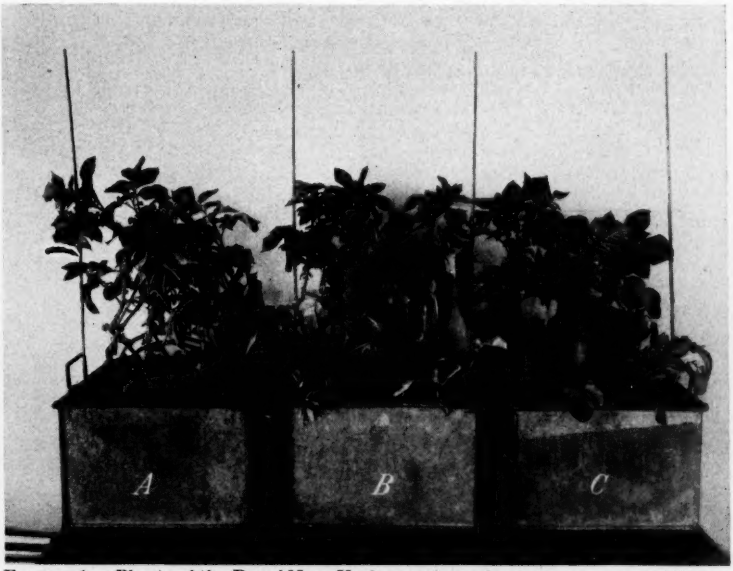

FIGURE 1.—Plants of the Rural New Yorker variety of potato, grown at different levels of potassium supply, showing symptoms of boron deficiency: *A*, 200 pounds; *B*, 400 pounds; *C*, 600 pounds of K_2SO_4 per acre, 1936.

the lower leaves. This latter effect was prominent in the Chippewa variety, which developed the least rolling of leaves. The symptoms produced on the leaves in this experiment closely resemble those found by Jones and Brown (*3, p. 123, pl. 5 B*) and others to be caused by a deficiency of boron. If so produced, they must represent a relatively mild deficiency, as death of the main stem tips and regeneration from lower branches, which will later be shown to attend an extreme lack of boron, were not observed. Only a limited application of boron was made directly (approximately 0.0002 pound per acre), and any additional supplies were provided by water, sand, and incidental sources. According to the findings of Carson,[6] the total boron added per culture in such indirect ways may have been at least as much as 0.0006 pound per acre. The improvement in appearance of leaves as the application of potassium was increased might have been due to

[6] CARSON, R. B. AN INVESTIGATION OF BORON IN RELATION TO POTATO DISCOLORATION. (Unpublished this is filed in the University of Wisconsin library.) 1939.

contamination of its salt with boron. Analysis of the writers' stock K_2SO_4 showed a content of 6.7 p. p. m. of boron, which would give a boron equivalent of 0.0034 pound per acre for the greatest application of this salt. This is about four times the amount accounted for above.

The crop was harvested on April 26 and tested May 8, which was probably too soon for the development of the post-harvest changes involved in the full expression of discoloration after boiling. The yields ranged from 320 to 635 gm. per pot, equivalent to 170 to 340 bushels per acre on the hill basis for clay loam. With the exception of the Rural New Yorker cultures to which calcium carbonate was supplied, none of the samples darkened appreciably after cooking, and even the Rural New Yorker would not have appeared discolored after peeling, as was subsequently discovered.

SEASON OF 1937

In the 1937 experiments emphasis was placed primarily on variation in the combination of the rarer mineral elements, superimposed upon a low (2.0 gm.) supply of potassium carrier. The combinations of the mineral nutrients are shown in table 6.

TABLE 6.—*Effect of various combinations of the rarer elements with a low supply of potassium on discoloration of potatoes grown in pots of sand or soil in the greenhouse, 1937*

Variety	Culture medium	Potassium supply	Rarer elements added [1]	Weight of fresh tubers [2]	Boiling record [3]
				Grams	
Chippewa	Sand	Low	Boron and manganese	435	W, W.
			Boron and copper	341	W, LG.
			Copper and manganese	118	W, W.
			Boron, copper, manganese.	337	W, W.
		Medium	None	110	W, W.
			do	92	W, W.
Rural New Yorker	do	Low	Boron and manganese	441	MG, W, W.
			Boron and copper	465	LG, W, W.
			Copper and manganese	172	W, W, W.
			Boron, copper, manganese.	354	W, LG, LG.
		Medium	None	140	W, W.
			do	117	LG, MG, W.
Burbank	Antigo clay loam		do	571	W, W.
			Boron	551	W, W.[4]
			Manganese	589	W, W.
			Boron and manganese	497	W, W.[4]
Irish Cobbler	Plainfield sandy loam.		None	387	W, W.[4]
			Boron	317	W, LG.
			Manganese	365	W, W.[4]
			Boron and manganese	315	W, W.[4]

[1] On Antigo clay loam and Plainfield sandy loam rare elements were applied with common nutrients.
[2] Total of duplicate cultures.
[3] Record of individual cultures: W=white; LG=light gray; MG=medium gray.
[4] Smallest tubers boiled light gray.

Soils from the northern and central (sandy) potato-producing areas were also planted to the Irish Cobbler and Burbank varieties respectively. The former had cooked white but was uncertified; the latter has been described in connection with the bed culture of 1937. Each of these soil cultures received 1.0 gm. of ammonium sulfate and monocalcium phosphate, 2.0 gm. of potassium sulfate, and either 0.5 gm. of hydrated manganous sulfate or 0.1 gm. of boric acid, or both.

Drying of the stem tips occurred in both varieties on sand, in association with lack of boron. There was also, as in the results of the previous year, a protective effect where potassium was liberally supplied to Rural New Yorker, as shown in figure 2. This effect might again be ascribed to an increase in boron. With the Chippewa there was serious bleaching of the main stem tips but relatively vigorous basal replacement growth. Both varieties developed essentially

FIGURE 2.—Plants of the Rural New Yorker variety of potato, given a liberal supply of potassium, showing the protective effect of boron: *A*, 400 pounds of K_2SO_4 per acre; *B*, 200 pounds of K_2SO_4 per acre; *C*, 200 pounds of K_2SO_4 plus B and Mn per acre. 1937.

normally on the low level of potassium supplemented by either boron and copper or boron and manganese.

The yields of the sand cultures show clearly the significance of boron in relation to the weight of tubers produced. As the boiling test was made about 4 weeks after harvesting, the results should have significance. The Chippewa variety was practically free of darkening after cooking while the Rural New Yorker was rather generally subject to this abnormality. The irregularity of discoloration of the Rural New Yorker variety in some treatments suggests variation in the character of the mother tuber as a possible cause. This seems a more probable cause of the irregularity than differences in the culture medium from pot to pot. Unless a questionable emphasis is placed upon the grayness of very small tubers, the soil cultures could be considered relatively free from discoloration.

SEASON OF 1938

In 1938 some of the plants were raised in glazed earthenware crocks holding 33 pounds of the silica sand. Various combinations of the rarer mineral elements were supplied as supplements to a lower level

(3 gm. of salt) of potassium as shown in table 7. A variation in iron supply was introduced by adding 7.5 times the usual amount of carrier with the fullest complement of rarer elements on the lower level of potassium. In the box cultures boric acid was applied in equivalents of 0.03, 0.3, and 3.0 pounds per acre, on each level of potassium.

TABLE 7.—*Yield and boiling records of potatoes produced with different combinations of the rarer elements and with different proportions between potassium and boron in greenhouse cultures, 1938*

POT CULTURE, ALL ON LOW POTASSIUM SUPPLY

Variety	Rare element added	Weight of fresh tubers [1]	Boiling record [2]
		Grams	
Irish Cobbler	Manganese, copper, zinc	112	W, W Sp sp
	Manganese, boron, zinc	197	W, W
	Manganese, boron, copper	170	W, W
	Manganese, boron, copper, zinc	156	W, W SP
	Boron, copper, zinc	193	W, W sp
	Manganese, boron, copper, zinc, high iron	172	W, W
Rural New Yorker	Manganese, copper, zinc	31	LG, LG SP, SP
	Manganese, boron, zinc	54	W, LG SP
	Manganese, boron, copper	36	LG, W
	Manganese, boron, copper, zinc	27	LG, W sp
	Boron, copper, zinc	[3] 30	W sp
	Manganese, boron, copper, zinc, high iron	[3] 45	LG

BOX CULTURE AT DIFFERENT K AND B LEVELS

	Level of K supply	Level of B supply		
Chippewa	Low	Low	247	W, W
		Medium	202	W, W
		High	264	W, W
	High	Low	158	W, W
		Medium	160	W, W
		High	174	W, W
Rural New Yorker	Low	Low	36	SP W, W
		Medium	102	W, W
		High	71	W, LG
	High	Low	[3] 17	LG
		Medium	53	LG, LG SP sp
		High		

[1] Sum of duplicate cultures except as noted.
[2] Record of individual cultures: W=white; LG=lighr gray; SP=spraing extensive ; sp=spraing limited.
[3] No tubers in 1 culture.

To minimize mineral contribution by the seed pieces, these were cut in the form of about 1-inch cubes. Transplanting of the Rural New Yorker stock was delayed 10 days because it was found deficient in root development. Although, like the other varieties, it was certified seed stock, its behavior in sprouting suggested that it was not normal.

Disturbances in vegetative development, which were first observed on March 20, were consistently related to cultural treatment. The pot cultures which received no boron showed withering and darkening of the tips of main stems and the leaves adjacent. Five days later the larger crown leaves were fading perceptibly, especially on the

Chippewa plants. This checking effect upon growth was followed by accelerated growth of the basal branches, which became conspicuous by March 30. In other cultures on the same level of potassium supply, marginal "burning" of leaves was associated with an application of only 3 pounds of boric acid per acre. Cultures not supplied zinc developed clusters of undersized youngest leaves in the Rural New Yorker variety, but this abnormality was not apparent in the Irish Cobbler. The high application of iron carrier was associated with abnormal upper leaves in the Rural New Yorker, these leaves being somewhat faded, brown at the margins, and rather stiff.

No anatomical abnormality of the conducting system, such as occurs in root nodules of leguminous species deprived of boron (*8*), was found in discoloring tubers.[7]

The Rural New Yorker produced small tops in the sand cultures in association with initial weakness of roots. On the used soils, replenished in major nutrients, they were thrifty but produced very small tubers.

The records of these crops are assembled in table 7. The potatoes stood for 1 month in an unrefrigerated storage room before they were boiled. The Irish Cobbler cooked a normal white, but in the raw state suffered from a form of internal brown spotting known as "spraing," or internal rust spot. The Rural New Yorker discolored more generally after cooking and was more subject to spraing. In both varieties the association of spraing with the boronfree mixture of nutrients was somewhat conspicuous. The high plane of iron salt in some of the cultures does not seem to have affected either the yield or the discoloration of tubers.

There was no discoloration of tubers of the Chippewa variety grown in boxes with different combinations of potassium and boron. On the other hand, several of the Rural New Yorker cultures in this series produced discoloring tubers, although without consistent relation to nutritional treatments. This variety also developed spraing, but again without any apparent relation to the nutrient supplied. There seems to be some correlation between soil-moisture content and the appearance of this form of internal browning. After March 20 the level of moisture at watering was decreased from 12 to 5 percent of the weight of dry sand, or essentially from 50 to 20 percent of saturation. Later the house became hot and the cultures lost water rapidly. These were desirable circumstances, as they closely simulate the weather conditions that aggravate discloration of the field crop after cooking. The water content of the sand in some of the crocks was found to be less than 3.0 percent. The severity of spraing developed in the Rural New Yorker variety under these conditions is shown in figure 3. It should be recognized that the driest cultures probably suffered the greatest elevation of temperature. This factor might either act independently or aggravate the effects of drought. From the irregularity of distribution of both darkening after boiling and spraing, it seems likely that extremes of drought and heat in some cultures contributed to these abnormalities. However, as suggested for previous crops, it is advisable to recognize peculiarities of the mother tuber as a possible factor in these responses.

[7] Examination was made by Elaine Tottingham under the direction of Prof. Emma L. Fisk of the Department of Botany.

SEASON OF 1939

The 1939 studies were devoted primarily to the role of seed stock in discoloration after cooking. It is not to be assumed that disease is the cause of discoloration, for in several examinations of commercial plantings the writers have found no association of blackening with symptoms either of yellow dwarf or leaf roll in the parent plant. Moreover, some recent results indicate that poor fertility of the soil and unfavorable weather conditions can induce postcooking discoloration of tubers from Rural New Yorker stock that is apparently normal.

Cultures in the iron boxes were exposed to heat when the development of the tubers was well under way. This was done by placing the boxes over the heating cable previously used in the bed planting. The maximum variation between heated and control cultures at midday in early April ranged from about 31° to 23° C. (88° to 73° F.), or

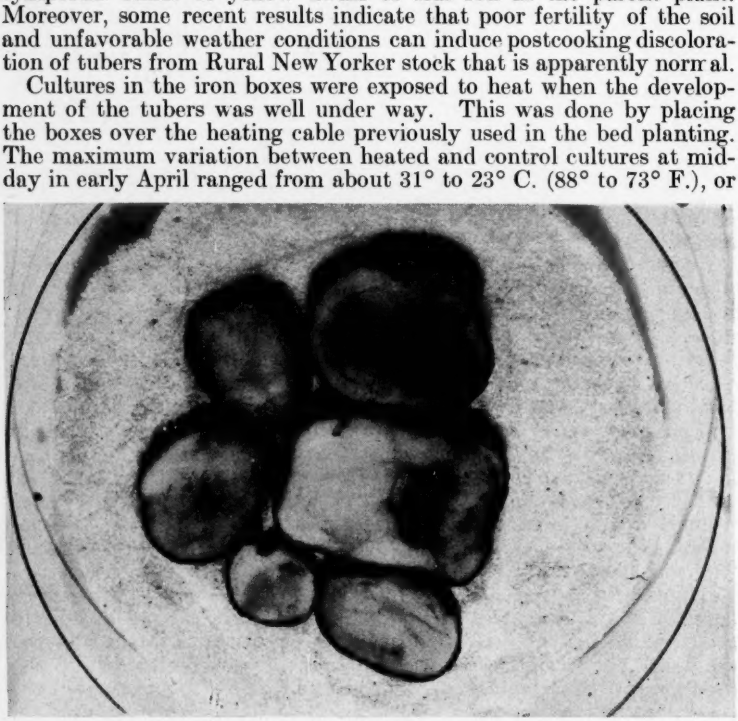

FIGURE 3.—Potatoes of the Rural New Yorker variety, grown in sand in which the moisture content fell below 3.0 percent, showing development of "spraing," or internal browning, 1938.

an interval of 8° C. (15° F.). By way of furnishing more favorable conditions for tuber development, cylindrical iron pots holding 63 pounds of sand were substituted for the smaller iron boxes in some of the experiments. Like the metal boxes these were coated on the inside with paraffin.

For the high-potassium level the fertilizer application in box cultures was equivalent to about 1,000 pounds of 3–20–23 per acre in field practice. One-half as much potassium was provided on the low level. All nutrients were increased 25 percent in the pot cultures commensurate with their increased size. The rarer nutrients were added in the amounts shown in table 5, except for the omissions of boron specified in table 8. These amounts appear small as compared with those supplied in field practice, but since they remain fully available, they should be adequate. The primary consideration was to

avoid toxicity resulting from an excess of these elements. The boron supply was added also at levels of 0.2 and 0.02 of the normal.

The seed stocks planted included tuber-test selections for either whiteness or discoloration after cooking and the presence of spraing. About 1 month after transplanting, the plants from discoloring seed stock were slender-stemmed and basally defoliated, and the crown leaves were small and crinkled. An examination of these plants by Dr. Russell Larson of the Department of Plant Pathology disclosed no symptoms of virus disease. Moreover, short growing tests in flats conducted under his guidance failed to reveal any such abnormality. About 10 weeks after planting, boron deficiency was partly compensated by the addition of 2.0 mg. of boric acid per culture, to aid tuber production in cultures showing injury from its omission. Three days later, selected cultures were restricted as to water supply, and these formed the subseries exposed to droughty conditions. The heating cable was operated from April 1 to 17. By April 3 some of the cultures in the heated group showed the desiccation of main stem tips characteristic of boron deficiency. Records of yield and discoloration are given in table 8.

TABLE 8.—*Yields and boiling records of potatoes grown in the greenhouse at different heat, moisture, and nutrition levels, showing influence of the seed stock on discoloration and the presence of spraing, 1939*

Seed stock	Soil moisture	Maximum soil temperature		Boron added	Weight of fresh tubers [1]	Boiling record [2]
	Percent	°C.	°F.		*Grams*	
Normal: Rural New Yorker A.	60	31	88	Yes	332	W, W, W, LG.
				No	300	W, W, W, W.
		23	73	Yes	254	W, W, W, W.
				No	283	W, W, W, W.
	20	31	88	Yes	317	W, W, W, LG.
				No	269	W, W, W, W.
		23	73	Yes	332	LG, LG, LG, W.
				No	223	W, W, W, W.
Discoloring: Rural New Yorker B.	60	23	73	Yes	128	W, LG.
	20	31	88	do	194	LG, LG.
Affected by spraing: Rural New Yorker B.	60	23	73	do	164	MG, LG. SP.
	20	31	88	do	142	MG, LG. SP.

Variation in nutrients	Potassium supply			
Apparently normal: Rural New Yorker D.	Low	None	53	W, W.
		Low	119	W sp, MG.
		Medium	141	LG, MG.
		High	133	LG, W.
	High	None	134	W, W.
		Low	104	W, W.
		Medium	220	W, W.
		High	112	MG, W.
Discoloring: Rural New Yorker B with spraing.	High N(NH_4)		196	LG, LG, LG. sp sp.
	Mixed N (NH_4 and NO_3)		219	LG, MG, MG. Sp sp sp.
	Low P		198	LG, LG, MG. Sp sp sp.
	Medium P		279	W, LG, LG. Sp sp.

[1] Sum of cultures recorded in boiling record.
[2] Record of individual cultures: W=white; LG=light gray; MG=medium gray; SP=heavy spraing sp=light spraing.

An examination of the results from heat and drought (table 8) shows little discoloration of the crop from normal Rural New Yorker seed stock. The most definite off color which occurred was under the combination of low soil temperature and low soil moisture, with boron added. This latter circumstance further indicates that blackening of cooked tubers is not due to lack of boron. It is worthy of note that spraing appeared to a limited extent under the particular combination of cultural conditions that was most definitely associated with darkening after cooking (table 8). In the present state of knowledge (*2*) it may be assumed that the phenol oxidases were unduly active in darkening tubers, with perhaps also an increased tissue content of either simpler phenolic compounds or tannins.

The irregular occurrence of discoloration within a given cultural treatment (temperature—moisture and potassium—boron series, table 8) is suggestive of individual variation either of environmental conditions in the pots or of a tendency to discolor transmitted by the mother tuber. In view of the close control of environmental factors, the results seem to indicate that the condition of the tuber planted is a primary factor in the development of blackening after boiling. Discoloration was rare and apparently independent of fertilizer additions in the series of crops grown from normal seed stock. On the other hand, crops grown from discoloring seed stock discolored in practically every instance. The use of nitrate in considerable quantities was attended with more serious discoloration than the use of ammonium salt only. However, in more recent work nitrogen has been supplied only as nitrate without resulting in the production of discoloring tubers.

Since potatoes grown under glass differ in respect to light and other environmental factors from those grown in the field, the results obtained in these greenhouse studies may not be entirely comparable to results obtained under field conditions. The nature of the metabolic disturbances associated with the blackening of potatoes after cooking has been covered by earlier papers from this laboratory (*7*, *5*, *2*). Apparently some related significance should also be given to the concept of Szent–Györgyi (*6*) that disease as well as mechanical injury conduce to the formation of melanin.

SUMMARY

A series of greenhouse studies has been conducted over a period of years to determine the cause of darkening in potatoes after boiling.

Different varieties of potatoes were grown on sandy soil in bed and pot cultures in the greenhouse during the winter and early spring. Different combinations of mineral nutrient elements were applied at different levels to the soil and to pure sand. Some of the plantings were subjected to differences in water content and temperature of the culture medium. The mature tubers produced were tested for discoloration after boiling, usually after unrefrigerated storage for 1 month.

The blackening of these crops after boiling depended most directly upon the record of the tubers planted. In agreement with observations on commercial crops, this abnormality appeared also primarily as a varietal characteristic. Discoloration was common in the varieties Rural New Yorker and Irish Cobbler but rare in Chippewa and Triumph.

Differences in the rates of supply of the major nutrient elements and of iron and boron did not affect darkening. Neither did the omission of manganese, copper, and zinc, other than as accidental constituents. Deficiencies of boron which brought about growth disturbances ranging from leaf roll to break-down of stem tips did not induce discoloration of boiled tubers.

Subjecting the developing tubers to heat, drought, or a combination of these factors, did not cause a consistent discoloration of the cooked tuber. "Spraing," or internal brown spotting, occurred in tubers subjected to less than 3.0 percent moisture in the sand, but this abnormality was not universally associated with blackening after boiling.

The stocks which produced discoloring crops were apparently free from the common potato diseases. However, since the tendency to discolor is inherited, an unrecognized virus or other disease may be present in such stocks.

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ENVIRONMENTAL, BREEDING, AND INHERITANCE STUDIES OF HYDROCYANIC ACID IN SORGHUM VULGARE VAR. SUDANENSE[1]

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INTRODUCTION

The Sudan grass acreage in Wisconsin has declined sharply since 1930. This decline may be attributed to a considerable extent to the occasional fatal poisoning of ruminants permitted to graze it. Poisoning of livestock is due to the presence in the plant of the cyanogenetic glucoside durrin, from which on hydrolysis and in the presence of an enzyme, hydrocyanic acid is released (*15*)[3]. Cyanogenetic glucosides are known to occur in a number of plant species, including white clover (*Trifolium repens*); common chokecherry (*Prunus virginiana*); sorghum (*Sorghum vulgare*). Johnson grass (*Sorghum halepense*); flax (*Linum usitatissimum*); arrowgrass (*Triglochin maritima*); common velvet grass (*Holcus lanatus*); and Christmasberry (*Photinia arbutifolia*).

A number of investigations of the factors associated with the development of hydrocyanic acid in Sudan grass have been reported. These investigations have been concerned primarily with the content of hydrocyanic acid in the plant at different stages of development and under various soil and climatic conditions. There is general agreement among workers that the content of hydrocyanic acid in Sudan grass and sorghum decreases as the plant approaches maturity, but results appearing in the literature do not agree as to the effect of edaphic and climatic factors on the hydrocyanic acid content of Sudan grass.

The primary objectives of the present investigations were (1) to determine the nature of the inheritance of the cyanogenetic glucoside durrin, (2) to develop strains of Sudan grass sufficiently low in this glucoside to materially reduce or eliminate entirely the danger of hydrocyanic acid poisoning in livestock, and (3) to determine the effect or various environmental factors on the hydrocyanic acid content of the plant so that due consideration could be given them in the studies on inheritance and inbreeding. In order to proceed with these studies it was necessary to have available a rapid, reasonably accurate, quantitative method for determining the hydrocyanic acid content so that large numbers of single plants could be analyzed during a short period of time.

[1] Received for publication September 15, 1942.

[2] The writers are indebted to Dr. O. S. Aamodt, under whose guidance the work was started, to Profs. R. A. Brink and L. F. Graber for continued interest and advice, and to Dr. Eisenhart, station statistician, for a variety of suggestions in connection with the presentation of the statistical evidence.

[3] Italic numbers in parenthesis refer to Literature Cited, 210.

REVIEW OF LITERATURE

A number of methods are available for determining the hydrocyanic acid content of plant material. Most of the methods could not be adapted to this study because of the large quantity of plant material required for the determination. Francis and Connell (*2*) used 50-gm. samples and Swanson (*14*) used 200 gm. when analyzing plant material by the prussian blue method of Viehoever and Johns (*16*). Boyd[4] using a modification of the alkaline picrate test of Guignard (*4*), analyzed samples of 1 gm. with a high degree of accuracy. Boyd's method provides for the liberation of the hydrocyanic acid by distillation in water to which a small amount of chloroform has been added. The procedure, as outlined by Boyd,[4] has been used extensively in this study whenever (1) sufficient plant material could be obtained, (2) the number of tests involved was not so great that time became a limiting factor, and (3) it was not necessary to evaluate populations of single plants which were to be used in the breeding work.

A rapid method for determining hydrocyanic acid in green tissues based on the picrate test was proposed by Pethybridge (*11*) in 1919. The use of chloroform in the test was suggested by Mirande (*9*), while Nowosad and MacVicar (*10*) later used toluene. The method consists of placing a small amount of plant material, cut into short pieces or macerated, in a test tube, adding a few drops of toluene or chloroform, and suspending a strip of moist filter paper saturated with sodium picrate solution above the mixture. The sodium picrate on the filter paper is reduced in the presence of hydrocyanic acid.

Nowosad and MacVicar (*10*) extracted the pigment from the test paper in 10 cc. of distilled water and compared it with standard color tubes similar to those used by Boyd. They concluded that the test was sufficiently accurate quantitatively for the selection of plants low in hydrocyanic acid. In this study the rapid method used was essentially that of Nowosad and MacVicar. However, the size of sample was reduced from 0.5 to 0.15 gm. of green plant material, and chloroform was used to release the hydrocyanic acid from the plant material. This procedure was used extensively for the determination of the hydrocyanic acid content of individuals of F_2 populations when the amounts of plant material available were small and when a large number of tests had to be made in a relatively short time.

Swanson (*15*), Willaman and West (*18*), Menual and Dowell (*8*), Boyd et al. (*1*), and Martin et al. (*6*) have reported that young sorghum plants and second growth contain more hydrocyanic acid on a unit-weight basis than do older plants. Franzke et al. (*3*) found 15 percent less hydrocyanic acid in second growth of sorghum than in the first growth. It has been shown by Martin et al. (*6*) that the hydrocyanic acid content of plant tissue of sorghum decreased as it became older. The lower internodes contained only one-third as much hydrocyanic acid as the uppermost internodes, and the leaves showed the same general trends. Swanson (*14*) has obtained essentially the same results.

Results appearing in the literature relative to the effect of differences in soil moisture on the hydrocyanic acid content of Sudan grass and

[4] BOYD, F. T. THE DETERMINATION OF THE FACTORS INFLUENCING THE AMOUNTS OF CYANIDE IN SUDAN GRASS. (Unpublished thesis on file in the University of Wisconsin library.) 1938.

sorghum are conflicting. Willaman and West (*19*) found that the hydrocyanic acid content of plants grown under inadequate moisture conditions was higher than that of plants grown under optimum moisture conditions. Swanson (*14*) concluded that hydrocyanic acid was more abundant in rapidly growing plants than in plants stunted by drought. Franzke et al. (*3*) have presented data to show that plants grown on soil with a low moisture content contained more than twice as much hydrocyanic acid as plants grown at a high level of moisture. Boyd [5] concluded that drought did not cause an increase in the amount of hydrocyanic acid present although the plants remained for a longer period in the stage where they contained a high level of hydrocyanic acid.

Menual and Dowell (*8*) found that the hydrocyanic acid content of Sudan grass was somewhat higher in the morning than in the afternoon. Boyd [5] obtained 30 percent more hydrocyanic acid at 1:00 p. m. than at 8:00 a. m. or 7:00 p. m. Franzke et al. (*3*) compared samples taken at 8:00 a. m. with those taken at 1:30 p. m. They concluded that there was slightly less hydrocyanic acid in the plant at 1:30 p. m. than at 8:00 a. m.

The effect of soil fertility on the hydrocyanic acid content of Sudan grass has been reported by several workers. Willaman and West (*18*) found that heavy nitrogen fertilization had no effect on the hydrocyanic acid content of Sudan grass under field conditions, except when the plants showed signs of nitrogen starvation. Maxwell (*7*) concluded that the nature of the soil had an important effect on the hydrocyanic acid content of Sudan grass. Boyd et al. (*1*) obtained sharp increases in hydrocyanic acid content with heavy applications of nitrogen. The soil used by Boyd was very low in nitrogen and the plants were chlorotic prior to fertilization with nitrogen. He found that soils deficient in phosphate produced plants high in hydrocyanic acid and that fertilization with phosphate reduced the level of hydrocyanic acid. Franzke et al. (*3*) concluded that applications of stall manure and phosphate reduced the hydrocyanic acid content of sorghum, while lime and nitrogen increased it. Manure, phosphate, and lime, however, did not produce as high a hydrocyanic acid content in plants as did lime alone.

EXPERIMENTAL TECHNIQUE AND MATERIAL

SAMPLING

The importance of developing a technique which would insure uniform sampling in testing individual plants for their content of hydrocyanic acid was recognized early in the study. The hydrocyanic acid content of seedling plants and of the second growth of Sudan grass are shown in table 1.

The average hydrocyanic acid content of plants in the seedling stage was 122 p. p. m., whereas that of the second growth was 224 p. p. m. The mean hydrocyanic acid content of the 40 inbred lines was 184 percent higher in second-growth material than in seedling plants. The data indicate that plants which were low in hydrocyanic acid in the seedling stage remained low in the second-growth stage (table 1, class 1), whereas the hydrocyanic acid content of plants which were relatively

[5] See footnote 4.

TABLE 1.—*Hydrocyanic acid content of seedling plants and of second growth of 40 inbred lines of Sudan grass* [1]

Class [2]	Seedling plants	Second growth	Class [2]	Seedling plants	Second growth
	P. p. m.	*P. p. m.*		*P. p. m.*	*P. p. m.*
1	35	31	5	266	456
2	73	89			
3	112	204	Average	122	224
4	123	342			

[1] Throughout the study hydrocyanic acid is reported as parts per million on a dry-matter basis.

[2] The lines which had been inbred for 3 to 7 generations were arranged in ascending order of their hydrocyanic acid content and then divided into classes of 8 lines each. Each quantity shown in the body of the table is the mean hydrocyanic acid content of the 8 lines in that class. Bulk samples from each of the inbred lines were analyzed for hydrocyanic acid by the Boyd method.

high, doubled from the seedling to the second-growth stage (table 1, classes 4 and 5). When Boyd's method was used for testing seedlings it was necessary to permit the plants to attain a height of not less than 18 inches before a sufficiently large sample for chemical analysis could be obtained. The plants were not uniform as to rate of growth so that a considerable range of development existed when all were ready to test. When the second growth was used, it was possible to obtain sufficient material a week after the plants were defoliated.

The date in table 2 were obtained under field conditions at Madison, Wis. in 1939. It is clear from these data that the hydrocyanic acid content of Sudan grass decreased as the plant increased in height and age. When various strains of Sudan grass are being evaluated for hydrocyanic acid, the material must, therefore, approximate the same stage of development.

TABLE 2.—*Hydrocyanic acid content of the second growth of a commercial and an inbred line of Sudan grass at various heights; Madison, Wis., 1939*

Height (inches)	Hydrocyanic acid content of second growth of—	
	Commercial Sudan grass	Inbred line
	P. p. m.	*P. p. m.*
3–4	670	310
6–8	250	100
12–14	150	60
20–24	70	50

The hydrocyanic acid content of various parts of Sudan grass plants growing under field conditions at Madison, in 1940 is summarized in table 3. It is clear from these data that young actively growing parts of the plant are significantly higher in hydrocyanic acid than the older parts of the plant.

Sudan grass growing in uncrowded nursery rows at Madison tillers until killed by frost. The data presented in table 3 would appear to indicate that reliable tests for hydrocyanic acid could be obtained by using only young leaves or young tillers even though the remaining parts of the plant were well advanced in growth. Extensive tests made during 1939 support this view. By using young tillers it was possible to obtain comparable plant material of various progenies

TABLE 3.—*Hydrocyanic acid content of various parts of the Sudan grass plant; Madison, Wis., 1940*

Plant part	Hydrocyanic acid content	
	P. p. m.	*Percent* [1]
Old stem	Trace	Trace
Young stem	20	0.9
Old leaves	30	10.7
Young leaves	760	67.6
Young tillers	760	20.7

[1] Percentage of total in plant.

throughout most of the growing period, unless tiller production was retarded by severe drought. Tillers from 5 to 7 inches in height were used. The sample for hydrocyanic acid analysis was taken from that portion of the tiller immediately below the uppermost leaf collar.

PLANT MATERIAL

The plant material serving as a basis for these studies was selected from a number of inbred lines which had been developed over a period of years at the Wisconsin Experiment Station. These stocks represent self-fertilized selections from seed obtained originally from Kansas, Texas, Oklahoma, Morocco, Australia, Argentina, Germany, and a number of commercial collections from local seed houses.

INFLUENCE OF ENVIRONMENTAL FACTORS ON THE HYDROCYANIC ACID CONTENT OF SUDAN GRASS

DROUGHT

Sudan grass plants were grown under drought conditions in the greenhouse at Madison during the winter of 1940–41. The temperature was maintained at 21° to 23° C. The plants were analyzed for their hydrocyanic acid content prior to the drought treatment and at 2-day intervals for 12 days during the period in which they were subjected to drought conditions. The test was continued until the soil-moisture level was below the permanent wilting coefficient. No water was added while the test was in progress. The data are summarized as follows, each figure representing the mean hydrocyanic acid content of six plants:

Days:	*P. p. m.*	Days:	*P. p. m.*
0	159	8	216
2	187	10	216
4	193	12	222
6	202		

The mean hydrocyanic acid content of the plants prior to the initiation of the drought treatment was 159 p. p. m., while that of the plants at the end of the experiment was 222 p. p. m. The data indicate that the hydrocyanic acid content of the plants increased to some extent as the moisture content of the soil decreased.

DIURNAL VARIATION

Six plants of known hydrocyanic acid content were grown under greenhouse conditions at Madison in the winter of 1940–41. The temperature was maintained at 21° to 23° C. Natural sunlight was

supplemented with artificial light from 200-watt Mazda bulbs to provide 15 hours of light daily. Single-plant tests for hydrocyanic acid were made every 3 hours for a period of 36 hours. The results of this study appear in table 4.

TABLE 4.—*Diurnal variation in the hydrocyanic acid content of Sudan grass.*

Plant No.	Time of test												Mean
	1 p. m.	4 p. m.	7 p. m.	10 p. m.	1 a. m.	4 a. m.	7 a. m.	10 a. m.	1 p. m.	4 p. m.	7 p. m.	10 p. m.	
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
1	400	420	360	420	340	420	320	400	350	380	430	400	387
2	100	160	150	150	150	110	150	180	300	160	300	220	178
3	280	180	520	200	460	530	360	600	400	380	380	570	405
4	1, 110	1, 320	980	1, 220	1, 380	800	600	970	1, 040	950	1, 460	1, 380	1, 101
5	920	760	400	780	740	710	680	1, 200	960	840	760	560	782
6	600	890	560	920	730	580	600	960	950	980	780	730	773
Mean	568	622	495	615	633	538	452	718	667	615	685	643	604

It is evident from the data in table 4 that there is no variation in hydrocyanic acid due to the diurnal factor. The data were analyzed statistically by several methods, and all failed to show any definite variation.

REGIONAL TEST

Further information concerning the relation of the hydrocyanic acid content of the plant to various environmental conditions was obtained after growing 10 lines of Sudan grass inbred from 3 to 7 generations at a number of agricultural experiment stations in the United States and Canada. The same 10 inbreds were grown in rod rows at Sturgeon Bay and Madison, Wis.; Ottawa and Saskatoon, Canada; Lincoln, Nebr.; State College, Pa.; College Station, Tex.; and Fort Collins, Colo., during the summer of 1939. The inbreds were cut back at the flowering stage and the second growth was harvested a week after the first growth had been removed. The second growth was dried and forwarded to Madison for analysis for hydrocyanic acid. Boyd's method was used in analyzing the material. From the data obtained (table 5) it would appear that the hydrocyanic acid content of Sudan

TABLE 5.—*Hydrocyanic acid in Sudan grass inbreds grown at various agricultural experiment stations in the United States and Canada in 1939*

Inbred	Ottawa	Madison, Wis.	Sturgeon Bay, Wis.	State College, Pa.	Saskatoon, Saskatchewan	College Station, Tex.	Fort Collins, Colo.	Lincoln, Nebr.
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
1	45	45	300	210	350	450	930	1, 070
2	40	55	45	150	175	380	950	930
3	30	30	120	50	220	140	930	650
4	45	35	140	90	250	350	700	930
5	120	140	370	320	480	650	1, 600	1, 950
6	50	110	180	200	280	500	1, 050	1, 070
7	80	80	120	365	480	1, 000	1, 300	1, 300
8	110	100	35	290	420	1, 050	1, 300	1, 950
9	100	120	360	500	530	1, 050	1, 600	2, 150
10	45	40	70	350	200	450	900	1, 300
Average	66. 5	75. 5	174	252. 5	338. 5	602	1, 126	1, 330

grass is profoundly influenced by variations in soil and climate. There was a significant difference in the hydrocyanic acid content of the same inbred lines grown at different experiment stations. In addition, there was a significant difference in the average hydrocyanic acid content of inbred lines used in the study at the different experiment stations.

The data were correlated with rainfall, temperature, and light conditions obtained from the Federal Weather Bureau at Madison, Wis. Average[6] maximum temperature for the month in which the samples were harvested gave a highly significant positive correlation coefficient of 0.96, which exceeds the 1-percent level of significance (0.92) for six pairs of observations. From this it is apparent that temperature plays an important part in determining the level of hydrocyanic acid. Rainfall and sunshine data were also correlated with the hydrocyanic acid content of Sudan grass. The correlation coefficients between rainfall and hydrocyanic acid and between sunshine and hydrocyanic acid were +0.3 and −0.12, respectively, neither of which approaches the 5-percent level of significance (0.83) for eight pairs of observations. Rainfall and sunshine have not shown a significant correlation with hydrocyanic acid content in these studies.

INHERITANCE STUDIES OF HYDROCYANIC ACID IN SUDAN GRASS

BREEDING METHODS

Some investigations of anthesis and pollination of Sudan grass were necessary before a breeding program could be attempted. Extensive investigations of this type with a number of sorghum varieties have been reported by Stephens and Quinby (*13*). They observed that flowering in most varieties occurred between 11 p. m. and 6 a. m., and that the time of maximum flowering varied from day to day. They pointed out that the normal flowering rhythm is controlled chiefly by temperature and light while humidity does not appear to be important. Pollen lost viability rapidly and no seeds were obtained when the pollen used was 5 hours old. The stigmas remained receptive for 8 to 16 days.

In the present experiments all emasculations were made by hand. Panicles of Sudan grass on which the terminal florets had just opened were selected for crossing. Panicles were permitted to start flowering in order to judge more nearly the age of the flowers to be emasculated. The tip of the Sudan grass panicle is weak and often breaks when an envelope is placed over it. For this reason the upper portion of the panicle was not used. The panicle was trimmed to from 40 to 50 florets which would open in from 18 to 42 hours. The emasculated panicle was covered at once with a glassine envelope 2¼×6 inches in size. The envelopes were held in place by small paper clips. The percentages of successful pollinations were found to be equally good under small and large envelopes.

Pollen was collected by placing parchment envelopes 3½×2×9 inches over a panicle that had started to flower the evening prior to pollination. The envelope was removed the next morning and sufficient pollen was usually obtained to pollinate 150 to 200 florets. A small camel's-hair brush was used to apply the pollen.

[6] The temperature correlation is based on 6 sets of data from all locations, except College Station, Tex., and Fort Collins, Colo.; that of rainfall and sunshine is based on 8 sets of data.

Sudan grass produces self-fertilized seed readily under either parchment- or kraft-paper envelopes. All types of selfing envelopes must be supported as the Sudan grass stem is too weak to bear a selfing envelope. Parchment envelopes 4×2½×11 inches were found to be most suitable for self-fertilizing Sudan grass. These envelopes were clasped about the stem by large paper clips and were supported on wooden stakes 1 inch×2 inches×8 feet. The stakes were driven securely into the soil beside the plant to be selfed and the envelopes were fastened to them.

During bright, warm weather at Madison, Sudan grass flowered early in the morning and by 8 a. m. flowering was usually complete. On cloudy, cool mornings flowering was delayed, the maximum number of flowers opening as late as 9 a. m. Pollen viability was found to agree closely with that reported by Stephens and Quinby (*13*) for sorghum. Approximately 3,000 hand pollinations were made at Madison in studying pollen viability. Pollen collected at 8 a. m. was applied to emasculated flowers at various times during the day. The summarized results of this study are given in table 6. It is evident from the data that the pollen loses its viability soon after it is shed.

TABLE 6.—*Seed set from hand pollinations made at various hours of the day*

Hour of pollination	Seed set	Hour of pollination	Seed set
	Percent		*Percent*
8 to 9 a. m.	73.12	11 to 12 a. m.	22.28
9 to 10 a. m.	45.91	2 to 3 p. m.	5.71
10 to 11 a. m.	22.13	3 to 4 p. m.	3.70

YEARLY VARIATION IN HYDROCYANIC ACID CONTENT

The hydrocyanic acid content of 175 inbred lines of Sudan grass present in the breeding nursery was determined in 1938, 1939, and 1940. The relative hydrocyanic acid content on a percentage basis for the same inbred lines for 1938, 1939, and 1940 was 100, 205.9, and 172.1, respectively. The correlation coefficient, r, between the 1938 and 1939 hydrocyanic acid content of the inbred lines involved in the study was $r=0.50$; between the 1939 and 1940 content, $r=0.52$; and between the 1938 and 1940 content, $r=0.66$, all of which greatly exceed the 1-percent level of r for 175 pairs of observations (0.19). These data show that there was a positive correlation between the hydrocyanic acid content of the inbred lines during the 3-year period. The results indicate that an inbred line which is high in hydrocyanic acid content in any given year will remain relatively high in other years, while an inbred which is low in hydrocyanic acid content in any given year will remain relatively low from year to year. These data afford proof that the ability of the plant to produce a particular level of hydrocyanic acid is an inherent characteristic. The differences which prevailed in the yearly levels were apparently due largely to differences in soil and climatic conditions during this period.

The second growth of 200 inbred lines appearing in the breeding nursery in 1939 and 44 inbred lines appearing in the nursery in 1940 was analyzed for crude protein and hydrocyanic acid. The purpose of this study was to determine whether any correlation existed between the crude protein and the hydrocyanic acid content of the plant. The

range in crude protein content between the inbred lines was from 18.63 to 26.63 percent in 1938 and 18 to 27 percent in 1939. The range in hydrocyanic acid content of the inbred lines was from 13.5 to 810 p. p. m. in 1938 and 10 to 1,300 p. p. m. in 1939. No definite positive correlation between the crude protein and hydrocyanic acid content was found to exist, although both characters were significantly influenced by environmental conditions.

EFFECTS OF INBREEDING ON YIELD

The amount of natural crossing which occurs in a species is of interest in any study of inbreeding. No determination of the amount of natural crossing in Sudan grass was noted in the literature. Sieglinger (*12*) reported natural crossing in sorghum to the extent of 5.38 percent. Karper and Connor (*5*) found 6.18 percent natural crossing between alternate rows of two types of sorghum.

The extent of natural crossing occurring between alternate rows of Sudan grass was determined at Madison in a study involving 870 plants. Open-pollinated seed from plants possessing a recessive glossy leaf character was chosen for the study. Counts were made of the seedlings that showed the dominant normal leaf color. The following percentages of natural crossing between rows were obtained: 4.8, 5, 9.4, 4.5, 8.2, 3, 7.6, 6.6, 10, and 7.7. The mean was found to be 6.7 $\pm$ 2.29 percent. These results agree closely with those cited above for sorghum.

Inbreeding in Sudan grass, according to Robertson (*17, p. 1066*), causes "no apparent loss of vigor after three generations of selfing," while Wenholz (*17, p. 1066*) reports "loss of vigor in some lines under continued inbreeding." At Madison it was observed that a fairly high degree of uniformity is reached in from 3 to 5 generations of inbreeding. In order to arrive at an estimate of the loss of vigor and the productive capacity of the most promising inbred lines, yield tests were conducted in 1939 and 1940. Quadruplicated plots of lines which had previously been inbred for from 3 to 7 generations were planted in a randomized block design. The same 20 inbreds were included in 1939 and 1940. Hay and seed yields were taken. The data were analyzed by the variance method, and are presented in table 7.

The yield of dry forage of 20 inbred lines tested in 1939 ranged from 2,710 to 4,451 pounds per acre. Commercial Sudan grass produced more dry forage than all except 1 of the inbred lines and was not significantly lower in productivity than this inbred line. The general mean of all inbred lines was 3,477 pounds of dry matter per acre. The average yield of hay of all of the inbred lines tested in 1939 was 82.2 percent that of commercial Sudan grass. Thirteen of the inbred lines produced 3,200 to 4,000 pounds of dry matter per acre and 3 produced more than 4,000 pounds per acre.

The dry forage harvested from the inbred lines in 1940 ranged from 2,445 to 3,913 pounds of dry matter per acre. Commercial Sudan grass was intermediate in yield. Eight of the inbred lines produced more dry forage than commercial Sudan grass although the yield of only three of the inbreds was significantly higher. The average yield of dry forage of all inbred lines tested in 1940 was 95.2 percent that of commercial Sudan grass.

TABLE 7.—*Yield per acre of dry forage and seed from various inbred lines and commercial Sudan grass in 1939 and 1940*

Inbred line No.	Yield of forage		Yield of seed	
	1939	1940	1939	1940
	Pounds	*Pounds*	*Pounds*	*Pounds*
3	3,970	3,622	739	933
7	2,923	3,119	837	808
10	4,000	2,460	847	720
12	2,751	3,529	508	1,113
20	3,311	2,871	525	893
23	3,116	3,289	607	1,106
26	4,451	3,522	1,224	936
45	3,620	2,927	815	688
51	3,778	2,833	766	964
53	3,472	3,799	629	1,294
62	3,394	2,986	599	1,099
65	3,207	3,664	741	1,312
70	2,816	3,041	516	595
74	2,710	3,388	561	921
81	3,166	3,812	604	957
106	3,579	3,607	882	1,511
128	3,676	3,196	820	1,170
134	4,076	3,913	1,124	1,014
145	3,499	3,435	1,020	1,170
153	4,032	2,445	573	890
Mean	3,477	3,273	746	1,004
Commercial Sudan grass	4,227	3,437	991	1,624

The yield of seed of the 20 inbred lines tested in 1939 ranged from 508 to 1,224 pounds per acre. Commercial Sudan grass yielded 991 pounds and ranked fourth in seed production. The majority of the inbred lines produced from 500 to 850 pounds per acre. The mean of the seed yields of all inbreds was 75.3 percent of the seed yield of the commercial Sudan grass. Seed yields were in general considerably higher in 1940 than in 1939. Commercial Sudan produced more seed than any of the inbred lines. The mean seed yield of all the inbred lines was only 61.8 percent that of commercial Sudan grass in 1940. It is concluded from these results that a loss in vigor accompanies inbreeding. This is particularly evident in seed production. It appears, however, that it may be possible to select inbred lines which are as vigorous as the parent stock even though the majority of the lines are inferior in yield. The decrease in seed production may have been due in part to the fact that emphasis was placed on forage quality rather than seed production in the selection of the inbreds. Even though this may be true, the decrease in ability to produce seed as a result of inbreeding is probably greater than the decrease in ability to produce vegetative growth.

DEVELOPMENT OF STRAINS OF SUDAN GRASS OF UNIFORM HYDROCYANIC ACID CONTENT

There were approximately 200 inbred lines of Sudan grass in the breeding nursery when the study was initiated in July of 1938. These lines had been inbred for from 3 to 7 generations and had been selected on the basis of morphological characters. Thirty-two crosses between plants selected on the basis of single-plant hydrocyanic acid tests were made during the summer of 1939. By this means the individual plants used in the cross were of known hydrocyanic acid content, and the distribution that occurred in the parent lines from which the plants

were selected could be observed. Two hundred F_1 plants of these crosses were grown in the greenhouse in the winter of 1939–40 and 2,000 F_2 plants in the field in the summer of 1940. Hydrocyanic acid tests were made on the individual F_2 plants by the modified method of Nowosad and MacVicar (*10*). The data summarizing this phase of the study are given in table 8.

TABLE 8.—*Distribution of F_2 plants on the basis of their hydrocyanic acid content,*[1] *1940*

Classification of parent plants according to hydrocyanic acid content	Distribution of F_2 plants according to whether hydrocyanic acid content [2] was—		
	Low	Medium	High
Low×low:	*Percent*	*Percent*	*Percent*
Total of all crosses	30.6	34.7	34.6
Cross producing largest percentage of low plants [3]	64	30	6
Cross producing smallest percentage of low plants [3]	5	25	70
Progeny producing largest percentage of low plants [4]	100	0	0
High×high:			
Total of all crosses	1.2	15.3	83.6
Cross producing largest percentage of low plants [3]	5	18.3	76.7
Cross producing smallest percentage of low plants [3]	0	1.6	98.4
Progeny producing largest percentage of high plants [4]	0	0	100
Commercial Sudan grass	7.5	27.5	65

[1] Plants were divided arbitrarily into classes on the basis of their hydrocyanic acid content. Plants containing 0–140 p. p. m. of hydrocyanic acid were considered low: 141–360 p. p. m., medium: and 361 p. p. m or more, high.
[2] Percent of total number.
[3] Includes all the progeny of a single cross.
[4] Includes all the F_2 plants of a single F_1 plant.

All except four of the F_2 progenies from crosses between parent plants both of which were low in hydrocyanic acid, segregated for this character. These four progenies appeared to be almost free of hydrocyanic acid. The cross yielding the largest percentage of low hydrocyanic acid plants produced 64 percent of its F_2 plants low in hydrocyanic acid, 30 percent intermediate, and 6 percent high. In the progeny of this cross with the largest percentage of plants low in hydrocyanic acid, 90 percent of the plants were low in hydrocyanic acid and 10 percent were intermediate. The progeny of this cross with the smallest number of plants low in hydrocyanic acid produced 40 percent plants low in hydrocyanic acid, 50 percent intermediate, and 10 percent high.

Crosses in which both parents were high in hydrocyanic acid produced almost no F_2 plants low in hydrocyanic acid. There was, however, considerable segregation in most of the combinations which were high in hydrocyanic acid. A number of plants appeared that were intermediate in hydrocyanic acid and occasionally one that was low. In the cross producing the highest percentage of F_2 plants high in hydrocyanic acid, less than 2 percent of plants intermediate in hydrocyanic acid appeared. All the plants were high in hydrocyanic acid in a number of the F_2 progenies.

A number of single-plant tests were made late in the summer of 1939 to determine the effectiveness of selection in commercial Sudan grass for the low hydrocyanic acid condition. Seedlings of plants that were believed to be low in hydrocyanic acid were grown in the field in the summer of 1940. The hydrocyanic acid content of the seedling plants (table 9) was determined by the single-plant test.

TABLE 9.—*Summary of results of one generation of single-plant selections for low hydrocyanic acid in commercial Sudan grass*

Item	Seedling plants with hydrocyanic acid content—		
	Low	Intermediate	High
	Percent	*Percent*	*Percent*
Total of all progenies	17.5	41.3	41.2
Total of 8 progenies with the lowest hydrocyanic acid content	32.5	45	22.5
Total of 8 progenies with the highest hydrocyanic acid content	2.5	37.5	60
Progeny lowest in hydrocyanic acid content	50	50	0
Original commercial sudan grass	7.5	27.5	65

The data presented in table 9 indicate that rapid progress can be expected in selecting for low hydrocyanic acid from commercial stocks of Sudan grass when the single-plant test for determining the hydrocyanic acid content of the plant is used. It is evident, however, from the data presented in tables 8 and 9 that a number of plants considered low in hydrocyanic acid were not homozygous for this character. Considerable segregation would be expected if a number of factors are involved in the inheritance of hydrocyanic acid.

Ten plants of each progeny were tested for hydrocyanic acid by the single-plant test. The more promising progenies were tested further to determine the degree of homozygosity existing with respect to hydrocyanic acid. Crosses were made between plants low in hydrocyanic acid from different lines. The F_2 plants were again analyzed individually and the procedure repeated. It has been possible by this means to develop strains low in hydrocyanic acid while retaining a high degree of vegetative vigor.

INHERITANCE OF HYDROCYANIC ACID

Inheritance studies involving the production of hydrocyanic acid in sorghum have been reported by Franzke et al. (*3*). They state that low hydrocyanic acid appears to be partly dominant and that one or two main factors and several minor modifying factors may be involved. Williams, (*20*), working with white clover, found cyanogenesis to be due to a single dominant factor, different levels of hydrocyanic acid being produced by modifying factors.

Eleven crosses between Sudan grass plants, high and low in hydrocyanic acid, were made during the summer of 1940. The F_1 plants were grown to maturity in the greenhouse in the winter of 1940–41. The F_2 seedlings were also grown in the greenhouse and were tested for hydrocyanic acid.

The F_1 plants were generally intermediate between the parents in hydrocyanic acid content. The average value of the F_1 of 32 crosses was very close to the expected intermediate value. The mean F_1 hydrocyanic acid content was 255.8 p. p. m., while the expected value was 253.9 p. p. m.

It was observed that the variability in hydrocyanic acid content in inbred lines of Sudan grass increased as a function of the hydrocyanic acid level present. The class interval was chosen so that at any level of hydrocyanic acid the standard deviation would cover about the same number of class intervals. The upper limit of any class x was

expressed by 21 x^2. Thus the class interval increased as a function of the class number squared. The resulting segregation in the F_2 and the variation of the parent plants are given in table 10.

TABLE 10.—*Frequency distribution of plants according to hydrocyanic acid content*

Cross No.	Parents and F_2 progeny	Distribution by class interval (upper limit) according to hydrocyanic acid content in p. p. m.										Mean hydrocyanic acid content
		21	84	189	336	525	756	1,029	1,344	1,701	2,100	
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*P. p. m.*
1	Parent 1	0	3	5								118
	Parent 2	0	0	0	0	1	7	1	1			669
	F_2	0	0	23	26	18	4					286
2	Parent 1	4	4	1								40
	Parent 2	0	0	0	0	5	3	1				530
	F_2	2	6	27	26	16	7	4	1			296
3	Parent 1	4	4	2								50
	Parent 2	0	0	0	0	1	7	1	1			669
	F_2	0	4	9	29	24	20	4	2			464
4	Parent 1	4	4	2								50
	Parent 2	0	0	0	0	6	3	1				553
	F_2	0	3	5	18	32	30	9	2			445
5	Parent 1	0	16	4								60
	Parent 2	0	0	0	0	6	3	1				530
	F_2	0	1	3	9	27	30	11	4			549
6	Parent 1	0	2	3	5							175
	Parent 2	0	0	0	0	6	3	1				519
	F_2	0	5	8	16	24	9	8	3			421
7	Parent 1	0	4	1	4							190
	Parent 2	0	0	0	0	1	2					566
	F_2	0	1	9	27	25	16	3				283
8	Parent 1	0	4	2	3	1						185
	Parent 2	0	0	0	0	6	3	1				519
	F_2	0	0	2	14	15	9	9				644
9	Parent 1	0	1	5	1	3						257
	Parent 2	0	0	0	0	1	2					566
	F_2	0	1	7	24	32	21	7	1			435
10	Parent 1	1	4	5								101
	Parent 2	0	0	0	0	0	0	2	3	2	1	1,267
	F_2	0	1	5	3	10	13	14	19	9	4	896
11	Parent 1	3	4	3								70
	Parent 2	0	0	0	0	0	0	2	3	2	1	1,267
	F_2	0	8	10	14	17	15	11	4	1		475
Total	Parent 1	16	50	33	13	4						
	Parent 2	0	0	0	0	33	33	11	8	4	2	
	F_2	2	30	108	206	240	174	80	36	10	4	

The data appearing in table 10 indicate that the distribution of the F_2 populations on the basis of their hydrocyanic acid content in most cases approaches the limits of both parents. In some populations the distribution reaches the extremes of both parents. In 5 of the 11 populations, the lower limit of the parent plant low in hydrocyanic acid is not reached by the F_2 distribution, while in 5 cases the upper limit of the high hydrocyanic acid parent was surpassed. Individual F_2 populations show considerable variation in distribution. The total F_2 distribution approaches the normal curve. These data indicate that the level of hydrocyanic acid is definitely inherited. It also appears from the F_1 and F_2 data that dominance is lacking. Nevertheless, the tendency for the F_2 distributions to be skewed to the right indicates that the genes concerned do not act in strictly additive fashion. From both breeding observations and F_2 data, there is evidence that more than a single pair of genes are involved in the production of hydrocyanic acid in Sudan grass. The exact number cannot be estimated from these data.

Crosses involving parents which were low in hydrocyanic acid were made to determine the effect of hybridity on the level of hydro-

cyanic acid in the F_2. The distribution of hydrocyanic acid in parts per million of the parents and F_2 populations of four crosses between parents low in hydrocyanic acid is reviewed in the histogram shown as figure 1. The data indicate that the hydrocyanic acid content of

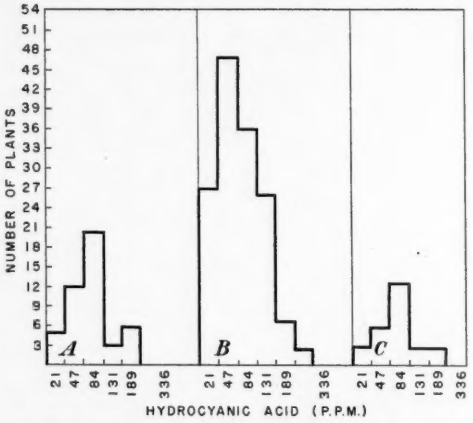

FIGURE 1.—Distribution of parent and F_2 populations of four crosses between plants low in hydrocyanic acid, based on hydrocyanic acid content: *A*, Parent population; *B*, F_2 population; *C*, parent population.

the F_2 plants was essentially the same as that of the parents. Three plants appeared with a hydrocyanic acid content somewhat higher than that of the parents, but there was no general tendency for the F_2 to be higher in hydrocyanic acid than the parents. The increased vigor of the F_1 and F_2 plants was not accompanied by an increase in hydrocyanic acid content. The results appear to indicate that vigor lost on selfing may be restored by hybridization without increasing the hydrocyanic acid content. However, further investigations including plants which are more variable in their hydrocyanic acid content than those used in these studies will be necessary before any generalization can be made relative to the effect of hybridization on the hydrocyanic acid content.

SUMMARY

The purpose of this investigation was (1) to determine the nature of the inheritance of the cyanogenetic glucoside durrin, (2) to develop strains of Sudan grass (*Sorghum vulgare var. sudanene*), so low in this glucoside as to materially reduce or eliminate entirely the danger of poisoning to livestock, and (3) to determine the effect of various environmental factors on the hydrocyanic acid content of the plant so that due consideration could be given them in the studies on inheritance and inbreeding.

Both the Boyd method and the method of Nowosad and MacVicar were found to be sufficiently rapid, accurate, and applicable to the conditions imposed by the study to warrant their use in determining

the hydrocyanic acid content of bulk lots and single plants of Sudan grass.

The second growth of Sudan grass contained approximately twice as much hydrocyanic acid as the first growth.

The hydrocyanic acid content of Sudan grass increased to some extent as the moisture content of the soil decreased. No variation in hydrocyanic acid attributable to the diurnal factor was observed. A highly significant positive correlation coefficient was found between temperature and the hydrocyanic acid content of the plants.

The hydrocyanic acid content of the plant tissue decreased as it became older. Meristematic parts of the plant were significantly higher in their content of hydrocyanic acid than the older parts of the plant. Young actively growing tillers were found to be uniformly and constantly high in hydrocyanic acid even though other parts of the plant were well advanced in growth. The hydrocyanic acid content of plants was determined by analyzing tillers 5 to 7 inches in height. By using young tillers it was possible to obtain comparable plant material throughout most of the growing period.

Flowering in Sudan grass was usually completed by 8 a. m. during bright, warm weather. The viability of the pollen was negligible 5 hours after it was shed. Sudan grass grown at Madison, Wis., was largely self-pollinated. Approximately 7 percent of natural crossing was found to occur.

The yield of forage and seed produced by 20 selected inbred lines was determined in 1939 and 1940. The average yield of dry forage produced by the inbred lines in 1939 and 1940 was 82.2 and 95.2 percent, respectively, that of commercial Sudan grass. The average yield of seed produced by the inbred lines in 1939 and 1940 was 75.3 and 61.8 percent, respectively, that of commercial Sudan grass.

One hundred and seventy-five inbred lines were tested for their hydrocyanic acid content in 1938, 1939, and 1940. The results indicate that an inbred line which is high in hydrocyanic acid in any given year will remain relatively high from year to year, while an inbred which is low in hydrocyanic acid in any given year will remain relatively low from year to year. No positive correlation was found to exist between the crude protein and the hydrocyanic acid content of the plant.

Vigorous strains of Sudan grass uniformly low in hydrocyanic acid were developed by hybridizing plants low in their content of hydrocyanic acid.

The hydrocyanic acid content of Sudan grass is not controlled by a single pair of genes. The hydrocyanic acid content of F_1 plants was intermediate between that of the parents. F_2 populations of plants from crosses between parents low in hydrocyanic acid were low in hydrocyanic acid. F_2 populations of plants from crosses between parents low and high in hydrocyanic acid resulted in a distribution extending to the limits of the parents with no tendency to be bimodal.

Vigor lost by selfing may be restored by hybridization without accompanying increases in hydrocyanic acid in the F_1.

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STAND IRREGULARITY AND ITS RELATION TO THE YIELDS OF SWEET CORN[1]

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INTRODUCTION

In the opinion of most canners and growers the yields of sweet corn are closely related to the uniformity of the field stand. Considerable care is taken to grade the seed into as many as 6 to 10 different sizes and to have available special planter plates for each seed size in order to assure a uniform drop. Having the proper equipment, seedsmen are often called upon to do the grading, a practice which they consider wasteful since buyers refuse to accept odd-sized grades and "round" kernels, which no planter plates will drop uniformly. Inasmuch as most of the sweet corn hybrids are single crosses it is virtually impossible to secure the uniformity of size and shape of seed associated with double-crossed field corn. Seedsmen's losses in grading, in addition to those associated with the regular cleaning process, frequently run as high as 10 percent, which, of course, adds considerably to the already high selling price.

It is self-evident that even with a uniform drop the field stand, because of natural hazards, will be more or less irregular. The tendency is to replant fields if the stands vary by more than one plant per hill on the assumption that this is absolutely necessary in order to secure maximum yields. The experiment herein reported was designed to determine the effect on yield of uniform stands as compared with irregular stands, including missing hills.

PLAN OF THE EXPERIMENT

The writer [2] has shown that under conditions at Urbana, Ill., the optimum planting distance for open-pollinated Country Gentleman sweet corn (*Zea mays*) is 40 by 40 inches with a stand of three plants per hill where weight is the primary consideration and four plants per hill where count is the important factor. Therefore, in the present experiment the planting distance of 40 by 40 inches in check rows was used with a maximum stand of four per hill.

The experiment was conducted at Urbana for the 3 years 1937–39 and included all the arithmetically possible stands between 0 and 4 per hill, allowing for a single variation in every other hill. There are 14 possible combinations, as follows:

0 and 1	1 and 1	2 and 2	3 and 3	**4 and 4**
0 and 2	1 and 2	2 and 3	3 and 4	
0 and 3	1 and 3	2 and 4		
0 and 4	1 and 4			

Since including stands of 0–1 and 1–1 would probably add but little experimental information, the lay-out was limited to the remaining 12 planting rates, 3 of which (2–2, 3–3, and 4–4) were uniform.

[1] Received for publication October 3, 1942.

[2] HUELSEN, W. A. YIELD OF SWEET CORN IN RELATION TO DISTANCE AND RATE OF PLANTING. Ill. Agr. Expt. Sta. Bul. 487, pp. 34–104, illus. 1942.

The plots having missing hills or irregular stands were planted so that in the odd-numbered rows, including border rows, the odd-numbered hills contained either the missing hill or the lower number of plants per hill. In the even-numbered rows, on the other hand, the sequence was reversed. This procedure assured an equal number of hills within plots for each of the two planting rates or for the respective missing hills and planted hills in irregular stands. In addition, the pattern was identical in all plots.

An experiment of this kind with only 12 variables lends itself well to a predetermined statistical treatment, and it was therefore laid out on the basis of a Latin square. Snedecor [3] presents a plan for a Latin square with 12 variables, and this was followed exactly in the field during the 3 years the experiment was conducted. However, precautions were taken to move the experiment to a new site each year, so that soil variations would be equalized.

Individual plots were laid out 6 rows wide by 12 hills long with an alley surrounding each plot. The day before harvest the outside row on all 4 sides of each plot was cut with a corn knife and removed. Thus the net plot actually harvested consisted of 4 rows, each 10 hills long, a total of 40 hills, as compared with the gross plot of 72 hills (6 rows, 12 hills each). It was considered that essentially all border effect was eliminated by this procedure. The net plot was 0.0102 acre in size.

Every possible effort was made to have the actual survival of plants at harvest time coincide with the indicated rate for each plot. The plots were planted at least twice as thickly as required and the seedlings were thinned to the proper rate in each hill when 4 to 6 inches high according to the pattern described above.

Illinois Country Gentleman Cross 8 × 6 was used throughout. The seed was obtained each year from an Illinois canning company specializing in the growing of its own seed. The seed was graded to the same uniform size each year and treated with the same dust disinfectant for control of seed-borne seedling disease organisms.

Variations in maturity between plots were relatively slight, but these were taken care of by harvesting each plot 21 or 22 days after the appearance of the majority of the silks, according to season.

All the ears, including undeveloped shoots, provided they showed silks, were harvested from each plot at the proper time, sacked, and brought to the laboratory. The unhusked ears were sorted into usable ears and culls. The former were then husked by machine and sorted again into prime husked ears and husked culls. No dented ears were found as the harvesting was completed before denting occurred. The prime husked ears were cut and scraped with a standard cream style cutter. Suitable counts and weights were taken at the various stages and the grading practices were similar to those followed by commercial canners in Illinois.

[3] SNEDECOR, G. W. CALCULATION AND INTERPRETATION OF ANALYSIS OF VARIANCE AND COVARIANCE. 96 pp. Ames, Iowa. 1934. (Iowa State Col., Div. Indus. Sci. Monog. 1.)

STATISTICAL METHODS

The methods of computing the annual yields were those mentioned by Snedecor [4] [5] for the variance of Latin squares. The method for determining the variances of the 3-year means was that of Fisher,[6] recommended by Cochran,[7] and shown in table 1.

In table 1 which represents the actual yields of prime cut corn on the plot basis (0.0102 acre) the sums of the squares for years were obtained by squaring the 3 annual plot totals, each representing the sum of the yields of 144 plots, and subtracting the correction factor consisting of the 3-year grand total divided by 3×144. Two degrees of freedom for years are allowed.

TABLE 1.—*Computation of the pooled variances of replicated Latin squares and subdivision of interaction basis of weight of prime cut corn on plot basis*

INTERACTION

Variations due to—	Degrees of freedom	Sums of squares	Mean square	Standard error
Years	2	5,173.70	2,586.85	
Type of planting	11	2,693.32	244.85	
Interaction	22	306.46	[1] 13.93	[1] 3.73
Total	35	8,173.48		
Tiers	33	1,391.70	42.17	
Columns	33	1,698.15	51.46	
Pooled error mean square	330	1,477.37	4.48	2.12
Total	396	4,567.22		

SUBDIVISION OF INTERACTION

Anomalous behavior of 44 plantings in 1938	1	93.72	93.72	
Remainder	21	212.74	10.13	3.18
Total	22	306.46	[1] 13.93	

[1] Interaction mean square and standard error.

The sums of squares for types of planting were obtained by adding the squares of the 12 3-year type-of-planting totals and subtracting the correction factor mentioned above. The sums of squares for tiers and columns were calculated by adding the respective sums of squares for each year.

The interaction mean square (years $\times$ type of planting) has been used instead of the pooled error mean square, following Cochran's suggestions. The former measures only the significance of those differences which have been consistent from year to year. Not only are plot errors measured, but also inconsistencies in behavior of the type of planting from year to year. Cochran has further suggested that the pooled error might be combined with the interaction mean square in instances where the latter is no higher than the former. However, the interaction mean square is higher in all cases except that of husked culls, recovery of prime husked ears, and recovery of

[4] See footnote 3, p. 212.

[5] SNEDECOR, G. W. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Rev. ed., 388 pp., illus. Ames, Iowa. 1938.

[6] FISHER, R. A. THE DESIGN OF EXPERIMENTS. 252 pp., illus. Edinburgh and London. 1935. (See pp. 211–215.

[7] Correspondence with W. G. Cochran, Statistical Laboratory, Iowa State College of Agriculture and Mechanical Arts.

prime cut corn. In all three instances the F values are not significant and, therefore, the planting rates do not differ significantly.

The differences required for significance at the 0.05 and 0.01 levels were determined from Fisher's table of t which was entered at 110 degrees of freedom for the annual means and at 22 degrees of freedom (types of planting × years) for the 3-year means.

EXPERIMENTAL RESULTS

GENERAL ANALYSIS OF DATA

The yields have been expressed in two ways, namely, the means and the percentages of the general mean. The former method shows wider differences in the high yielding year 1938 than in 1937 or 1939, the range from highest to lowest for weight of prime cut corn alone in table 4 being 642, 1,106, and 745 pounds per acre for 1937, 1938, and 1939, respectively. The annual changes in the range of differences undoubtedly contribute to the types of planting × years interaction. For the same years in table 4 the percentage differences of prime cut corn are much more stable, being respectively 39.15, 46.85, and 45.38.

The experimental treatments fall into three main groups which may be studied either separately, or collectively, as follows: (1) Alternate hills completely missing; (2) increasing planting rates in uniform stands; (3) irregular stands, all hills planted. Judged, however, on the basis of the yields of the most important yield components (weights of sorted unhusked ears, prime husked ears, and prime cut corn) in tables 2, 3, and 4, the first group is the only one distinctly separate, inasmuch as the three treatments, 0–4, 0–3, and 0–2 are consistently the lowest producers. Groups 2 and 3 fail to show any distinctly

TABLE 2.—*Actual and relative acre weights of sorted unhusked ears of Country Gentleman sweet corn under specified variable and uniform planting rates and distributions for the 3-year period, 1937–39*

Type of planting	1937			1938			1939			3-year average		
	Annual mean yield	Percent of general mean	Rank	Annual mean yield	Percent of general mean	Rank	Annual mean yield	Percent of general mean	Rank	Yield	Percent of general mean	Rank
	Tons			*Tons*			*Tons*			*Tons*		
0–2	2.148	77.43	12	2.597	70.28	12	1.973	70.97	12	2.239	72.62	12
0–3	2.268	81.76	11	3.021	81.76	11	2.204	79.28	11	2.498	81.02	11
0–4	2.329	83.96	10	3.199	86.58	10	2.431	87.44	10	2.653	86.05	10
1–2	2.816	101.51	8	3.528	95.48	9	2.548	91.65	9	2.964	96.14	9
1–3	2.976	107.28	4	3.716	100.57	8	2.723	97.95	8	3.138	101.78	8
1–4	2.902	104.61	6	3.871	104.76	6	2.834	101.94	7	3.202	103.86	6
2–2	2.915	105.08	5	3.885	105.14	5	3.060	110.07	4	3.286	106.58	5
2–3	2.790	100.58	9	3.723	100.76	7	3.010	108.27	5	3.174	102.95	7
2–4	3.079	110.99	2	4.247	114.94	3	3.143	113.05	3	3.490	113.20	2
3–3	3.068	110.60	3	3.920	106.09	4	3.274	117.77	1	3.420	110.93	3
3–4	3.110	112.11	1	4.313	116.72	2	3.155	113.49	2	3.526	114.37	1
4–4	2.884	103.97	7	4.318	116.86	1	3.009	108.24	6	3.404	110.41	4
General mean	2.774			3.695			2.780			3.083		
F value	**16.77			**31.43			**23.21			**21.69		
Standard error	.0818	2.95		.0951	2.57		.0853	3.07		.0892	2.89	
Difference required for significance:												
At the 1 percent level	.3034	10.94		.3527	9.53		.3164	11.39		.3556	11.53	
At the 5-percent level	.2292	8.27		.2665	7.20		.2391	8.60		.2616	8.49	

**=highly significant.

separate tendencies. In other words, evenness of stand is not necessarily the principal criterion of production.

TABLE 3.—*Actual and relative acre weights of prime husked ears of Country Gentleman sweet corn under specified variable and uniform planting rates and distributions for the 3-year period, 1937–39*

Type of planting	1937			1938			1939			3-year average		
	Annual mean yield	Percent of general mean	Rank	Annual mean yield	Percent of general mean	Rank	Annual mean yield	Percent of general mean	Rank	Yield	Percent of general mean	Rank
	Tons			*Tons*			*Tons*			*Tons*		
0–2	1.445	75.34	12	1.908	70.12	12	1.291	72.37	12	1.548	72.30	12
0–3	1.541	80.34	11	2.183	80.23	11	1.411	79.10	11	1.712	79.96	11
0–4	1.569	81.80	10	2.341	86.03	10	1.563	87.61	10	1.824	85.19	10
1–2	1.949	101.62	8	2.589	95.15	9	1.617	90.64	9	2.052	95.84	9
1–3	2.078	108.34	4	2.766	101.65	7	1.756	98.43	8	2.200	102.76	8
1–4	2.014	105.01	5	2.820	103.64	6	1.817	101.85	7	2.217	103.55	6
2–2	2.000	104.28	6	2.865	105.29	5	1.941	108.80	4	2.269	105.98	5
2–3	1.934	100.83	9	2.748	100.99	8	1.926	107.96	5	2.203	102.90	7
2–4	2.147	111.94	3	3.129	114.99	3	2.026	113.56	3	2.434	113.68	2
3–3	2.159	112.56	2	2.902	106.65	4	2.128	119.28	1	2.396	111.91	3
3–4	2.192	114.29	1	3.200	117.60	2	2.031	113.85	2	2.474	115.55	1
4–4	1.987	103.60	7	3.202	117.68	1	1.901	106.56	6	2.363	110.37	4
General mean	1.918			2.721			1.784			2.141		
F value	**18.08			**33.63			**20.42			**18.04		
Standard error	.0603	3.14		.0701	2.57		.0578	3.25		.0706	3.30	
Difference required for significance:												
At the 1-percent level	.2237	11.65		.2600	9.53		.2144	12.05		.2814	13.14	
At the 5-percent level	.1689	8.80		.1965	7.20		.1620	9.11		.2070	9.67	

**=highly significant.

TABLE 4.—*Actual and relative acre weights of prime cut kernels of Country Gentleman sweet corn under specified variable and uniform planting rates and distributions for the 3-year period, 1937–39*

Type of planting	1937			1938			1939			3-year average		
	Annual mean yield	Percent of general mean	Rank	Annual mean yield	Percent of general mean	Rank	Annual mean yield	Percent of general mean	Rank	Yield	Percent of general mean	Rank
	Pounds			*Pounds*			*Pounds*			*Pounds*		
0–2	1,244	75.85	12	1,682	71.24	12	1,215	73.99	12	1,380	73.37	12
0–3	1,338	81.59	11	1,914	81.07	11	1,305	79.48	11	1,519	80.75	11
0–4	1,344	81.95	10	2,048	86.74	10	1,440	87.70	10	1,610	85.59	10
1–2	1,672	101.95	7	2,239	94.83	9	1,507	91.78	9	1,806	96.01	9
1–3	1,755	107.01	4	2,379	100.76	7	1,625	98.96	8	1,920	102.07	8
1–4	1,705	103.96	6	2,438	103.26	6	1,669	101.64	7	1,937	102.98	7
2–2	1,709	104.21	5	2,446	103.60	5	1,793	109.20	4	1,983	105.42	5
2–3	1,668	101.71	8	2,363	100.08	8	1,788	108.89	5	1,939	103.08	6
2–4	1,853	112.99	2	2,725	115.42	3	1,855	112.97	2	2,144	113.98	2
3–3	1,846	112.56	3	2,537	107.45	4	1,960	119.37	1	2,115	112.44	3
3–4	1,886	115.00	1	2,768	117.24	2	1,850	112.67	3	2,168	115.26	1
4–4	1,662	101.34	9	2,788	118.09	1	1,697	103.35	6	2,049	108.93	4
General mean	1,640			2,361			1,642			1,881		
F value	**14.27			**28.26			**16.16			**17.58		
Standard error	56.8	3.46		64.7	2.74		57.8	3.52		60.8	3.23	
Difference required for significance:												
At the 1-percent level	210.7	12.83		240.0	10.16		214.4	13.06		242.4	12.89	
At the 5-percent level	159.2	9.70		181.3	7.68		162.0	9.86		178.3	9.48	

**=highly significant.

In most instances the yields rank in much the same relative order from year to year, but in the case of the weights of sorted unhusked ears, prime husked ears, and prime cut corn, discrepancies in the yields of certain treatments must be considered. The weights of sorted unhusked ears, prime husked ears, and prime cut corn in tables 2, 3, and 4, respectively, are unusually consistent with reference to each other within single years as indicated by the relative rankings. Comparisons of rankings from year to year show that the various rates of planting are consistent with the three exceptions discussed below.

The 1–3 rate of planting ranked uniformly fourth in 1937, varied from seventh to eighth in 1938, and was uniformly eighth in 1939.

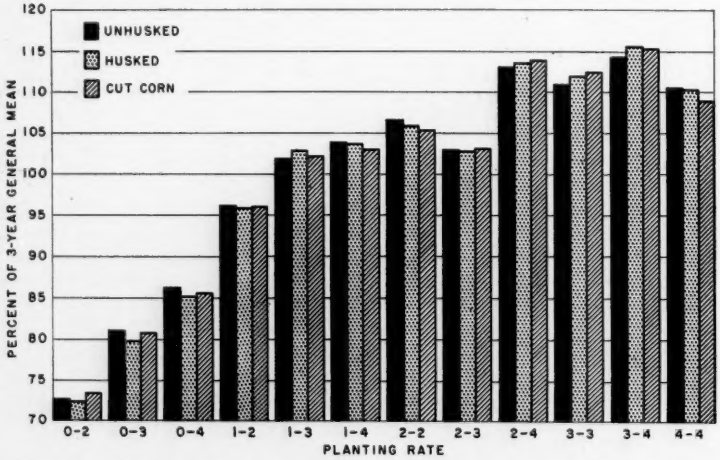

FIGURE 1.—Relation between type of stand and 3-year mean yields of sweet corn expressed as percentages of the general mean in a Latin square, plotted from data in tables 2, 3, and 4. Significant differences at the 5-percent level are 8.49 percent for weights of sorted unhusked ears (*A*), 9.67 percent for prime husked ears (*B*), and 9.48 percent for prime cut corn (*C*).

The data in tables 2, 3, and 4 show, however, that in 1937 the differences between fourth and eighth rankings are not significant. Therefore, the 1937 discrepancy is of minor importance.

The 2–3 planting rate in tables 2, 3, and 4 ranks uniformly fifth for 1939, which is considerably higher than the eighth to ninth ranking in 1937 and the seventh to eighth in 1938. The differences between fifth and eighth ranking in 1939 are significant, but not in 1937 and 1938. However, figure 1 shows that the 2–3 rate has an unduly low 3-year mean yield, indicating a depression of yield in 1937 and 1938 rather than an unusual increase in 1939. Inasmuch as the differences between the fifth and eighth rankings in 1937 and 1938 are not significant, the discrepancy cannot be considered a true anomaly.

The 4–4 rate of planting, however, presents a true anomaly, inasmuch as it varied from seventh to ninth rank in 1937, was uniformly sixth in 1939, but reversed its trend to reach first in 1938. Undoubt-

edly this anomalous performance has a biological explanation. The general means in tables 2, 3, and 4 show that 1938 was an exceptionally favorable year as compared with 1937 and 1939. Consequently, under optimum conditions a heavy rate of planting such as 4–4 might give exceptionally high yields, reverting to a much lower level of production under less favorable conditions. In other words, the 4–4 planting rate may be considered a borderline treatment, requiring exceptional conditions to produce the maximum weight of ears. That the 4–4 rate will produce a large number of ears even under average conditions has been demonstrated by the writer [8] and is also shown by the data from this experiment in table 6. The number of unhusked and husked prime ears per acre ranked first in 1938 and ranged from second to third in 1937 and 1938, but without any significant differences between first and third ranks in 1937 and 1938.

In other words, the ears from planting rate 4–4 merely developed to larger size in 1938, and the weights per unhusked ear averaged 94.00 percent of the general mean in 1938 as compared with 84.62 and 91.84 percent in 1937 and 1939, respectively. Similarly, the weights per prime husked ear averaged 94.61 percent in 1938, 85.79 percent in 1937, and 91.52 percent in 1939. In all 3 years treatment 4–4 had uniformly the lightest weight ears.

The reversal of planting rate 4–4 from low ranking in 1937 and 1939 to first in 1938 so far as weights of sorted unhusked ears, prime husked ears, and prime cut corn are concerned, contributes to the treatments $\times$ years interaction. The interaction may be subdivided, following Snedecor [9] in Section 15.9, as shown in table 1. The method consisted of multiplying each of the 3 yearly totals of 12 replications of treatment 4–4 by 12 and subtracting the respective yearly total yields of the 144 plots in the experiment. The 3 differences thus obtained were calculated according to the following:

$$\frac{[(2\times 1938 \text{ difference}) - (1937+1939 \text{ differences})]^2}{22\times 432=9504} =$$

mean square for treatment 4–4 in 1938.

This product was subtracted from the interaction mean square of years $\times$ type of planting.

The ratios between the mean squares for the single degree of freedom and the 21 degrees of freedom as shown in table 1 give the following F values:

Weight of sorted unhusked ears	8. 37
Weight of prime husked ears	10. 49
Weight of prime cut corn	9. 25

All of these are highly significant.

EFFECT OF STAND IRREGULARITIES ON YIELDS OF PRINCIPAL EAR COMPONENTS

Weights of Sorted Unhusked Ears, Prime Husked Ears, and Prime Cut Corn

The percentages of the general means from tables 2, 3, and 4 have been plotted in figure 1. The treatment sequence used in plotting figure 1 is the same as that which appears in the tables. According to this sequence the yields increase with a marked degree of regularity

[8] See footnote 2, p. 211.
[9] See footnote 5, p. 213.

through the first seven planting rates, that is, as far as rate 2–2. Beyond this point there is considerable irregularity. It should be noted that all three components of yield are in remarkably close agreement.

More complete comparisons were possible by plotting the data from tables 2, 3, and 4 in five groups holding one of the alternate pairs of hills constant and permitting the second to vary as follows:

		2–0	3–0	4–0
		2–1	3–1	4–1
0–2	1–2	2–2	3–2	4–2
0–3	1–3	2–3	3–3	4–3
0–4	1–4	2–4	3–4	4–4

These groupings involved 21 rates of planting. Since there were only 12 rates in the experiments, 9 were used twice. Three of the 5 groups included uniform rates of planting. If uniformity of planting had a strong influence on yields, the uniform rates in each of the three groups unhusked ears, husked ears, cut corn—should have given unusually high yields, but this did not prove to be the case.

The data for the three yield components show that thickness of stand rather than uniform planting rate is the dominant factor affecting yield. In this respect the results are very similar to those of Kiesselbach and Weihing,[10] who compared systematically variable stands with uniform stands of field corn over a 14-year period and found that the difference averaged only 0.1 bushel per acre.

The data are all very consistent in showing the dominant effect of stand. The 2–3 planting rate is the only one disturbing the regularity. The reasons for this disturbance have already been discussed.

The data in tables 2, 3, and 4 show that the five highest ranking rates on the basis of 3-year mean yields are 3–4, 2–4, 3–3, 4–4, and 2–2 in the order given. In the case of weights of sorted unhusked ears and prime husked ears, all five of these rates have yield differences less than the 5-percent level of significance, but on the basis of prime cut corn weights in table 4, the 2–2 rate has a barely significant lower yield than 3–4, the highest in the group. However, owing to the anomalous behavior of treatment 4–4 in 1938, the interaction may be subdivided and 4–4 removed from consideration in comparing other rates. Using 21 degrees of freedom, the differences required for significance at the 5-percent level between the 3-year means drop to 7.47, 8.20, and 8.24 percent, respectively, in tables 2, 3, and 4. On this basis the 2–2 rate is significantly lower than the top ranking 3–4 rate for all three yield components, but at the same time is not significantly higher than the next heavier rate 2–3.

This is the only point in the experiment excluding the missing hill rates where there is the slightest indication that a uniform rate may be superior to a corresponding irregular rate, that is, one having the same number of hills per plot. However, further analysis in table 5 fails to show that the 2–2 rate is superior because of its uniformity.

The present experiment is complicated by the fact that two variables must be considered. The first of these, uniformity of stand, has already been discussed. The second, number of plants per acre, has not been considered separately. For each pair of hills the planting rate varies from two to eight by single steps. In other words there are seven

[10] KIESSELBACH, T. A., and WEIHING, R. M. EFFECT OF STAND IRREGULARITIES UPON THE ACRE YIELD AND PLANT VARIABILITY OF CORN. Jour. Agr. Res. 47. 399–416, illus. 1933.

different thicknesses of planting in the experiment. The weights of the principal ear components are arranged in table 5 on the basis of number of plants in each pair of hills. Four of the seven groups contain more than one type of plant distribution.

The 0–3 and 0–4 rates are consistent in being significantly lower than any of the other plantings within their respective groups. Apparently where an irregular stand consists of a missing hill it falls into an entirely different category than an irregular stand having all hills planted, even if there is only one plant in the irregular hill. The remaining rates within each group fail to differ significantly in weight of any of the three principal yield components. This indicates that stand irregularity fails to produce significant differences in weights of sorted unhusked ears, prime husked ears, and prime cut corn when the total number of plants per unit of area is held constant. In other words when thickness of stand is held constant, irregularity has no significant influence on yields, but the missing hill type of irregularity causes significant decreases.

TABLE 5.—*Effects of irregularities within classified stand groups on the yields of sweet corn* [1]

Number of plants per 2 hills	Rate	Yield				
		Weight of unhusked ears [2]	Weight of prime husked ears [2]	Weight of prime cut corn [2]	Number of sorted unhusked ears [2]	Number of prime husked ears [3]
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
2	0–2	72.62	72.30	73.37	69.34	69.16
3	0–3	81.02	79.96	80.75	78.43	77.21
	1–2	96.14	95.84	96.01	91.98	92.00
4	0–4	86.05	85.19	85.59	85.98	85.78
	1–3	101.78	102.76	102.07	98.29	99.15
	2–2	106.58	105.98	105.42	103.13	102.44
5	1–4	103.86	103.55	102.98	105.73	105.72
	2–3	102.95	102.90	103.08	102.23	102.29
6	2–4	113.20	113.68	113.98	115.07	115.17
	3–3	110.93	111.91	112.44	111.06	112.27
7	3–4	114.37	115.55	115.26	117.80	118.66
8	4–4	110.41	110.37	108.93	120.96	120.10

[1] Expressed in percentages of the general 3-year mean.
[2] Significant differences at the 5-percent level are 8.49 for weight of unhusked ears, 9.67 for weight of prime husked ears, and 9.48 for weight of prime cut corn, but in the comparisons which do not involve the 4–4 rate the significant differences are 7.47, 8.20, and 8.24 respectively.
[3] Significant differences at the 5-percent level are 5.66 for number of sorted unhusked ears, and 6.34 for number of prime husked ears.

NUMBER OF SORTED UNHUSKED AND PRIME HUSKED EARS

In experimental work dealing with sweet corn, the yields in terms of number of ears per acre must also be considered since truck growers sell by count and not by total weight as cannery growers do. The yields on the basis of numbers of ears are summarized in table 6.

The general trends of yields expressed as number of sorted unhusked and prime husked ears are very similar to those shown when these yields were expressed by weight except for the fact that the 4–4 planting rate is the highest and its behavior was consistent during the 3 years of the experiment. This agrees with the previous work of the writer [11] in which it was shown that the four-per-hill rate is the one to use for Country Gentleman where maximum number of ears

[11] See footnote 2, p. 211.

TABLE 6.—*Actual and relative 3-year mean acre yields of Country Gentleman sweet corn in terms of number of sorted unhusked and prime husked ears, weight per sorted unhusked and prime husked ear, and weight of unhusked culls planted under specified variable and uniform rates and distributions*

Type of planting	Ears—						Weight—						Unhusked culls		
	Sorted unhusked			Prime husked			Sorted unhusked			Prime husked					
	Per acre	Percent of general mean	Rank	Per acre	Percent of general mean	Rank	Per ear	Percent of general mean	Rank	Per ear	Percent of general mean	Rank	Weight per acre	Percent of general mean	Rank
	Number			*Number*			*Pound*			*Pound*			*Tons*		
0-2	6,503	69.34	12	6,240	69.16	12	0.6861	104.52	1	0.4919	104.42	1	0.207	46.73	12
0-3	7,355	78.43	11	6,966	77.21	11	.6764	103.05	4	.4861	103.18	3	.283	63.88	11
0-4	8,063	85.98	10	7,739	85.78	10	.6544	99.70	7(8)	.4672	99.17	8	.366	82.62	10
1-2	8,626	91.98	9	8,300	92.00	9	.6856	104.45	2	.4889	103.78	2	.373	84.20	9
1-3	9,218	98.29	8	8,945	99.15	8	.6747	102.79	5	.4853	103.01	5	.437	98.65	8(7)
1-4	9,915	105.73	5	9,538	105.72	5	.6425	97.88	9	.4589	97.41	10	.437	98.65	7(8)
2-2	9,672	103.13	6	9,242	102.44	6	.6769	103.12	3	.4858	103.12	4	.443	100.00	6
2-3	9,587	102.23	7	9,229	102.29	7	.6575	100.17	6	.4722	100.23	6	.509	114.90	4
2-4	10,791	115.07	3	10,391	115.17	3	.6417	97.76	10	.4619	98.05	9	.505	114.00	5
3-3	10,415	111.06	4	10,129	112.27	4	.6544	99.70	8(7)	.4708	99.94	7	.539	121.67	3
3-4	11,047	117.80	2	10,706	118.66	2	.6328	96.40	11	.4558	96.75	11	.590	133.18	2
4-4	11,344	120.96	1	10,835	120.10	1	.5936	90.43	12	.4283	90.91	12	.625	141.08	1
General mean	9,378			9,022			.6564			.4711			.443		
F value	**68.51			**56.93			**.0891			**.0961			**8.92		
Standard error	181	1.93		195	2.16		.0089	1.36		.0059	1.25		.041	9.26	
Difference required for significance:															
At the 1-percent level	721	7.69		777	8.61		.03548	5.41		.02352	4.99		.1634	36.88	
At the 5-percent level	531	5.66		572	6.34		.02610	3.98		.01730	3.67		.1202	27.13	

**Highly significant.

per acre is the primary consideration. Accordingly, larger yield differences may be expected as a result of the greater effect of total stand. This is what actually occurred. In the case of yields in terms of number of sorted unhusked ears, the 4–4 and 3–4 rates are significantly the highest in the entire experiment and do not differ statistically. On the basis of yields of number of prime husked ears, there are three statistically identical rates which are significantly higher than all the remaining rates, namely, 4–4, 3–4, and 2–4. Here, again, uniformity of stand is not the criterion of yield.

Further analysis of the data on number of sorted unhusked and prime husked ears in table 5 shows that the missing-hill type of stand irregularity falls into a category different from that of the irregular stands in which all the hills are planted, inasmuch as the yields are significantly lower than the rest of the treatments in their respective groups. In fact the latter show no significant differences.

Weight Per Sorted Unhusked and Prime Husked Ear

The summarized 3-year mean weights per ear shown in table 6 are obviously influenced primarily by number of plants per hill, decreasing inversely with respect to number of plants per hill. None of the decreases are statistically significant, however, except where the planting distribution included four plants per hill.

The data may be grouped and analyzed in the same manner as table 5, but not shown here. Significant differences failed to appear, with one exception, when the data were analyzed on such a basis, indicating that when the number of plants was held constant in pairs of hills, their distribution had no significant effect on weight per ear, although there was a distinct tendency toward a decrease where the group member included a hill with four plants. In one of the groups, namely, 0–4, 1–3, and 2–2, the 0–4 member produced significantly smaller prime husked ears than the highest member, 2–2.

Recovery of Prime Husked Ears and Prime Cut Corn

To the canner any changes in the percentages of prime husked ears and prime cut corn recovered from unhusked ears are of obvious importance. The data for these components have been computed, but as the F values fall considerably below significance, it may be assumed there is no difference between the rates of planting. These conclusions are in agreement with the results of the writer,[12] who found that the recovery of prime husked ears in the Country Gentleman variety was not significantly changed by increased rates per hill except where the number of plants per hill was large and the planting distances close. In such instances the recovery of prime husked ears decreased. However, both the rates per hill and the distances between rows were beyond the limits of the present experiments.

Weight of Unhusked and Husked Culls

The yields of culls are of no particular importance except for the fact that their presence is highly objectionable to the canner because of the added cost of handling and of waste disposal. The yields of unhusked culls summarized in table 6 proved to be, with the exception

[12] See footnote 2, p. 211.

of the 2–2 and 3–3 rates, remarkably uniform from year to year on the basis of the relative rankings. In contrast, the annual yields of husked culls were, with few exceptions, exceedingly divergent.

The weights of unhusked culls increase at a rapid rate in relation to the number of plants per hill, but these increases are independent of regularity of stand. Comparisons made in the same manner as in table 5, but not shown, indicate that there are no statistical differences within the four groups containing more than one rate of planting. As a matter of fact, in two of these four groups (0–4, 1–3, and 2–2; 2–4 and 3–3), which are the only ones containing uniform planting rates, the uniform rates have greater weights of unhusked culls than the irregular rates both with and without missing hills.

The weights of husked culls have F values considerably below significance. It is assumed, therefore, that neither thickness nor irregularity of stand shows significant differences. The fact that thickness of stand fails to increase the weights of husked culls within the limits of the present experiment is in agreement with the work of the writer,[13] who obtained significant increases only in plantings heavier than those tested here.

COMPARISON BETWEEN UNIFORM AND MISSING HILL STANDS

Comparisons on the basis of the general mean have been made between uniform and irregular rates of planting, the latter including stands with alternate missing hills. The effect of alternate missing hills on yields may be considered separately on the basis of comparisons with the corresponding uniform planting rate and ignoring the general mean. Three pairs of treatments may be compared, namely, 2–2 and 0–2, 3–3 and 0–3, 4–4 and 0–4. These comparisons are summarized in table 7. The reductions in the total yields due to missing hills are progressive, being lowest where the stand is four per hill and highest where it is two per hill. Weight per ear is not only greater in missing hill stands, but also shows a progressive tendency in relation to rate opposite to that of total yields.

TABLE 7.—*Changes in 3-year mean yields due to alternate missing hills in 3 stands of sweet corn* [1]

Yield components	Change due to missing hills in indicated stand		
	2	3	4
Weights:[2]	*Percent*	*Percent*	*Percent*
Sorted unhusked ears	**31.86	**26.96	**22.06
Prime husked ears	**31.78	**28.55	**22.81
Prime cut corn	**30.41	**28.18	**21.42
Unhusked culls	**53.27	**47.50	**41.44
Number:[2]			
Sorted unhusked	**32.76	**29.38	**28.92
Prime husked	**32.48	**32.21	**28.57
Weight per ear:[3]			
Sorted unhusked	1.36	*3.36	**10.24
Prime husked	1.26	**3.25	**9.08

[1] Yields are expressed as percentages calculated from the formula $\frac{a-b}{a} \times 100$ where a and b are the 3-year mean yields of the respective complete and alternate missing hill stands.

[2] All figures given are decreases.

[3] All figures given are increases.

*Significant at 5-percent level.

**Significant at 1-percent level.

[13] See footnote 2, p. 211.

MATURITY IN RELATION TO STAND

The writer has previously shown [14] that increasing the number of plants per hill tends to delay maturity. Such a tendency was also noted in the present experiment. Data for 2 of the 3 years, 1937 and 1938, are shown in table 8. These data were computed from silk counts taken every other day during the early maturation period. Methods of making such counts and their interpretation in terms of maturities are discussed by Huelsen and Michaels.[15] Owing to the 2-day intervals between counts it proved to be impractical to interpret maturities in terms of time units. The data in table 8 have, therefore, been expressed as percentages above or below the theoretical number of silks (75 percent of the total) at midsilking for each planting rate on the day the entire experiment actually reached midsilking. The 2–4 rate may be used as an example. The silks of the 2 center rows (total of 20 hills) were counted, a procedure uniformly followed. The 20 hills of the 2–4 rate contained 60 plants, or a total of 720 plants in 12 replications. It was assumed that 75 percent of 720 plants, namely, 540, would be the number of silks showing at the midsilking point. The validity of this procedure is discussed by Huelsen and Michaels. Since 540 silks was the theoretical midsilking point and 556 were actually counted on August 15, 1938, the midsilking point of the entire experiment, the percentage was calculated as follows:

$$\frac{556}{540}\times 100 = 102.96 \text{ percent.}$$

The 2–4 rate had 2.96 percent more than 75 percent of the theoretical number of silks showing on August 15 and, therefore, was very slightly earlier than the entire experiment.

TABLE 8.—*Relation between maturity of sweet corn and type of stand* [1]

1937

Number of plants in alternate (varying) hill	Number of plants per hill, stand constant				
	0	1	2	3	4
0			65.56	40.74	−1.74
1			51.11	30.28	−.67
2	65.56	51.11	45.28	3.78	2.22
3	40.74	30.28	3.78	11.48	−5.24
4	−1.74	−.67	2.22	−5.24	−25.69

1938

0			48.89	28.52	8.61
1			25.92	20.28	−6.22
2	48.89	25.92	42.22	−4.67	2.96
3	28.52	20.28	−4.67	−.93	−6.20
4	3.61	−6.22	2.96	−6.20	−25.42

[1] Expressed as percentage increase (earlier maturity) or decrease (later maturity) in relation to theoretical number of silks at midsilking.

[14] See footnote 2, p. 211.

[15] HUELSEN, W. A., and MICHAELS, W. H. THE YIELD COMPLEX OF SWEET CORN. Ill. Agr. Expt. Sta. Bul. 432: pp. 505–608, illus. 1937.

The data in table 8 show a close relationship between maturity and stand, delayed maturity being associated with increased planting rates. There is no conclusive evidence that uniform planting rates have any effect on maturity independent of total stand.

SUMMARY

The effect of irregularities in the field stands of hybrid Country Gentleman sweet corn has been studied over a 3-year period. Twelve combinations of rate and distribution of planting were included, 3 of them uniform, 3 with missing hills, and 6 with a constant rate of 1, 2, 3, or 4 plants in one hill and the next one varying. The 12 stand variations were laid out in the field on the Latin-square basis with 12 replications. The experiment was moved to a new site each year.

Total number of plants per acre rather than irregularity of distribution within reasonable limits was the factor which determined yields. The yield components thus affected were number and weight of sorted unhusked ears, number and weight of prime husked ears, and weight of prime cut kernels.

Weight per unhusked and prime husked ear was influenced primarily by thickness of planting and not by irregularities in stand. Increasing the number of plants per hill reduced the weight per ear.

Recovery of prime husked ears and of prime cut corn proved to be highly variable from year to year and showed no significant trends within the limits of this experiment either with respect to total stand, or irregularities of stand.

Weights of unhusked culls increased rapidly in relation to total stand, but were not affected by irregularities of distribution. Weights of husked culls failed to show any definite trends either with respect to total or irregular stands.

A comparison of yields from uniform stands containing two, three, and four plants per hill with those from equivalent half stands in which alternate hills were missing, showed that significant reductions occurred in all components of yield except weight per ear where significant increases were noted in most cases. Both the decreases and increases also showed distinct trends. Total weights of ears, cut corn, and unhusked culls along with total number of ears all showed the greatest decreases in missing hill stands containing 2 per hill and the smallest for four per hill. This trend was reversed in the case of the weight per ear increases.

Increasing the total stand tended to delay maturity as determined by silk counts. There was no consistent relationship between maturity and irregularities in stand.

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